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Department of Zoology, University of Kerala
Kariavattom, Trivandrum, India 695581

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The Visionary and Guiding Force Behind this .....

PROF. K K NAYAR
(1920 – 1975)

From the Editors Desk .....

Twenty-five years is a fairly long period of time for a scientific journal for its uninterrupted publication. ENTOMON started in 1976 as a biannual journal for publishing scientific research papers on insect science on behalf of the association for Advancement of Entomology (AAE) with the vision and efforts of late Prof. K. K. Nayar, late Prof. M. R. G. K. Nair, late Prof. N. R. Prabhoo and Prof. V. K. K. Prabhu, has now grown into a quarterly journal recognized as probably the only journal on insect science which is being published from India which keeps up its regularity as well as scientific quality of any international scientific journal. On the occasion of this Silver Jubilee year of publication of ENTOMON, I am happy to bring forth this particular issue by according due recognition and acknowledgement to the entire Editorial Board and Referees (both Indian and overseas) who have always been patient enough in meticulously reading through the articles judging the quality of scientific research findings to be published in ENTOMON. I have always tried to keep up the esteem of this Journal, both in its quality as well as in its layout ever since I inherited its Editorship from Prof. V. K. K. Prabhu in 1995. In this process I am sure I would have displeased many of the authors and I hope I may be excused for this non-deliberate act.

D. Muraleedharan
Managing Editor
ENTOMON

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Inducible Antibacterial Proteins in Haemolymph of the American Bollworm, *Helicoverpa armigera* (Hübner)

S. Subramanian and G. T. Gujar*

Division of Entomology, Indian Agricultural Research Institute, New Delhi 110012, India
Email: gtgu_ent@iari.ernet.in; gtgujar@yahoo.com

ABSTRACT: Studies on the antibacterial activity in the haemolymph of the American bollworm, *Helicoverpa armigera* (Hübner) were carried out in the fifth instar larvae immunized with live bacteria. Inoculation of the fifth instar larvae with bacteria immediately led to their clearance from haemolymph and induction of lysozyme activity. Lysozyme activity of the immune haemolymph was 5623 ng/5 μl in terms of chicken egg white lysozyme as compared to sham injected or saline injected control that showed only 10 ng/5 μl of lysozyme content. Lysozyme content was highest in fifth instar larvae than in pupae or in young larvae/earlier instars. The haemolymph of the immune larvae showed highest antibacterial activity to *Bacillus megaterium*, (with a least killing time of 13.3 min) to be followed by *Bacillus subtilis*, *Escherichia coli* DH5α and *E. coli* NM522. *Bacillus thuringiensis* var. *kurstaki* was resistant to the immune haemolymph. Acidic polyacrylamide gel electrophoresis of the haemolymph of immune larvae showed that at least 10 major proteins (polypeptides) with molecular weights ranging from 14 to 77.5 kDa are produced *de novo* in response to bacterial inoculation. Of these, 2 proteins appeared to be closely related to lysozymes on the basis of their electrophoretic mobility with respect to chicken egg white lysozyme. The importance of immune proteins for protection of host insect against bacterial infection is discussed.

KEYWORDS: Antibacterial activity, lysozyme activity, immune proteins, American bollworm, *Helicoverpa armigera* (Hübner).

INTRODUCTION

Insects that have evolved over millions of years have lived in a hostile environment of pathogenic and nonpathogenic microbes. In the process, they have acquired an unique defence system. In In the process, they have acquired an unique defence system. In response to invading microbe(s), insect defence is composed of two-stage strategy; first one, haemocytic to be followed by a humoral. The haemocytic defence is mainly by phagocytosis, encapsulation and nodulation of a foreign matter by phagocytes *viz.*,

*Corresponding author

plasmatocytes and granular haemocytes. The details of these mechanisms are reviewed by Götz and Boman (1985) and Ratcliffe (1993). The humoral response that succeeds the cellular one takes time, in view of *de novo* synthesis of antibacterial proteins in the haemolymph. Unlike vertebrate's synthesis of specific immunoglobulins, these antibacterial proteins are non-specific in sequestering the remaining foreign matter of different kinds. Engström (1992) and Gillespie *et al.* (1997) reviewed insect immunity with particular emphasis on the antibacterial proteins. A number of antibacterial proteins have been isolated and characterized from many insect species. These include lysozymes, cecropins, attacins, haemolins, diptericins, defencins, apidaecins, abaecins, drosocins, andropins, coleoptericins, ceratotoxins and others (cf. Hetru *et al.*, 1998). As many as 15 antibacterial proteins have been characterised from different species of lepidopterans, and some of these proteins share a homology in many respects.

The American bollworm, *Helicoverpa armigera* Hübner is an important key pest of agricultural crops all over the world. It is polyphagous in nature. Very little information is available on the immune proteins in this insect species. In the present paper, we describe the isolation and characterization of antibacterial proteins in *H. armigera*.

MATERIALS AND METHODS

Test insects

Insects used in the present investigations were obtained from the laboratory colony maintained on gram flour based artificial diet at 27 °C, 60% RH, 14L: 10D. The composition of artificial diet was essentially similar to that of Nagarkatti and Prakaash (1974). The castor semilooper, *Achaea janata* were collected from the field and reared on castor leaves under hygienic conditions as per conditions used for *H. armigera*.

Bacterial strains and culture techniques

Different bacteria used in the study include *Escherichia coli* strains, DH5α, JM83, NM522; *Bacillus megaterium*, *Bacillus polymixa*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus thuringiensis* var. *kurstaki* and lyophilized cells of *Micrococcus lysodeikticus* (Sigma). Overnight cultures of bacteria in liquid broth at 31 °C were centrifuged and washed with lepidopteran saline and used for immunization of fifth instar larvae of *H. armigera*.

Insect immunization and collection of haemolymph

Approximately 10^6 colony forming units (cfu)/ml of live bacteria suspended in lepidopteran saline were injected into larvae @ 2 μl/insect between prolegs with sterile needle attached to I ml syringe with the help of electrically operated microapplicator (ISCo, Lincoln, Nebr. USA), after anaesthetizing them with carbon dioxide. After injection, larvae were spot-sterilized with 70% ethanol to avoid secondary infection. Larvae, which were bleeding after injection, were discarded. Insects were immunized with bacteria *viz.*, *E. coli* NM522, JM83, DH5α and *B. subtilis*. Haemolymph was

collected from insects 48 h after immunization in a precooled microcentrifuge tube, centrifuged for 10 min at 2000 rpm at 0 °C and stored at −70 °C until use.

Clearance of bacteria from haemolymph of test insect

Last instar larvae of *H. armigera* were injected with *E. coli* @ 10^4–10^6 cfu/each. Insects were bled at hourly interval and after serial dilution with extraction buffer, the bacterial titre was estimated by plate assay after incubation at 37 °C for 24 h.

Estimation of lysozyme activity

Lysozyme activity was estimated by nephalometry and inhibition zone assay. In the former, a dried cell wall suspension of *M. lysodeikticus* in 0.1 M phosphate buffer (pH 6.5) and of concentration that gave 0.2–0.3 absorbance at 570 nm was incubated for 30 min at 37 °C in presence of 10 μl of lysozyme or haemolymph sample of immunized insect. After incubation, lytic reaction was stopped by placing suspension on an ice-bath and the absorbance estimated again at 570 nm. The lysozyme activity (units), a ratio of decrease in absorbance to that of sample, is estimated as per Hultmark *et al.* (1980).

In case of inhibition zone assay, 6 ml of 1% agar in 0.1 M phosphate buffer (pH 6.4) containing 1 mg/ml of *M. lysodeikticus* cells was uniformly spread over in a Petriplate and the wells of 2.5 mm diameter punched. The test haemolymph samples from the immune insect were then added in each well and incubated at 30 °C over night. The diameter of clear zone of inhibition around the well depended upon the lysozyme activity of the sample. The standard curve of diameter of inhibition zone and lysozyme concentration was prepared with varying concentrations of lysozyme and used for estimation of lysozyme content in the immune haemolymph samples as per Hultmark *et al.* (1980).

Antibacterial assay

Antibacterial activity of the immune haemolymph was estimated by killing and inhibition zone assay. In killing assay, the immune haemolymph was mixed with bacterial suspension, 2×10^4 cfu/ml, in 0.1 M phosphate buffer (pH 6.5) in 1 : 10 v/v proportion and the samples were drawn at various time intervals. The samples were uniformly spread upon nutrient agar medium, incubated overnight and then colonies counted. Time of killing for different bacteria, when no colonies developed, was estimated as per Hultmark *et al.* (1980).

In the inhibition zone assay, the antibacterial activity of immune haemolymph was estimated similar to inhibition zone assay for lysozyme described above; except for the use of bacteria other than *M. lysodeikticus*.

Protein estimation

The plasma samples were estimated for their protein content using bovine serum albumin V as a standard at 610 nm as per Lowry *et al.* (19951).

Acidic polyacrylamide gel electrophoresis (acidic PAGE)

Electrophoresis of immune haemolymph was carried out in 15% polyacrylamide gels at pH 4.0 using a discontinuous buffer system (Gabriel, 1971; Hultmark *et al.*, 1982). After the electrophoretic run, the gels were stained and destained as suggested by Steck *et al.* (1980).

Sodium dodecyl sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed with LKB electrophoresis apparatus at 6–15% gradient gel. Electrophoresis was carried out as described by Maniatis *et al.* (1990). Gels were stained either with Coomassie Brilliant Blue R-250 or silver stained as per Blum *et al.* (1987). Two SDS gels were run with different gradients to resolve high and medium weight proteins. For the purpose of estimation of their molecular weights, the standard molecular weight marker kits namely Sigma SDS 70L kit and Promega midrange molecular weight markers were used.

Partial purification of immune haemolymph

The immune haemolymph samples were partially purified as described by Hultmark *et al.* (1980). The immune haemolymph sample was purified as follows. Pooled immune haemolymph sample is chromatographed on to Sephadex G-100 (pH 5.1) and purified fractions were assayed for antibacterial activity and characterized by acidic PAGE. The active fraction was then further chromatographed on to CM Sepharose and eluted with ammonium acetate pH 5.0 of increasing molarity from 0.15 to 1.0 M and rechromatographed with ammonium formate pH 6.6 with increasing molarity of 0.1 to 0.6 M.

Statistical analyses

Data were subjected to students' t test and other appropriate statistical analyses wherever needed by using a computer software (Indostat CS, Hyderabad). Estimation of molecular weights was done by comparing relative mobility with standard markers using QPRO.BAS PC software.

RESULTS

Clearance of bacteria from haemolymph

The concentrations of viable bacteria present in the haemolymph of fifth instar larvae at various times following injection of *E. coli* DH5α increased up to 12 h of inoculation and then drastically declined [Fig. 1(a)]. The decline of bacterial count below the base level dose (10^6 cfu) was at 48 h postinjection, with the lowest count of 475 cfu at 80 h after injection.

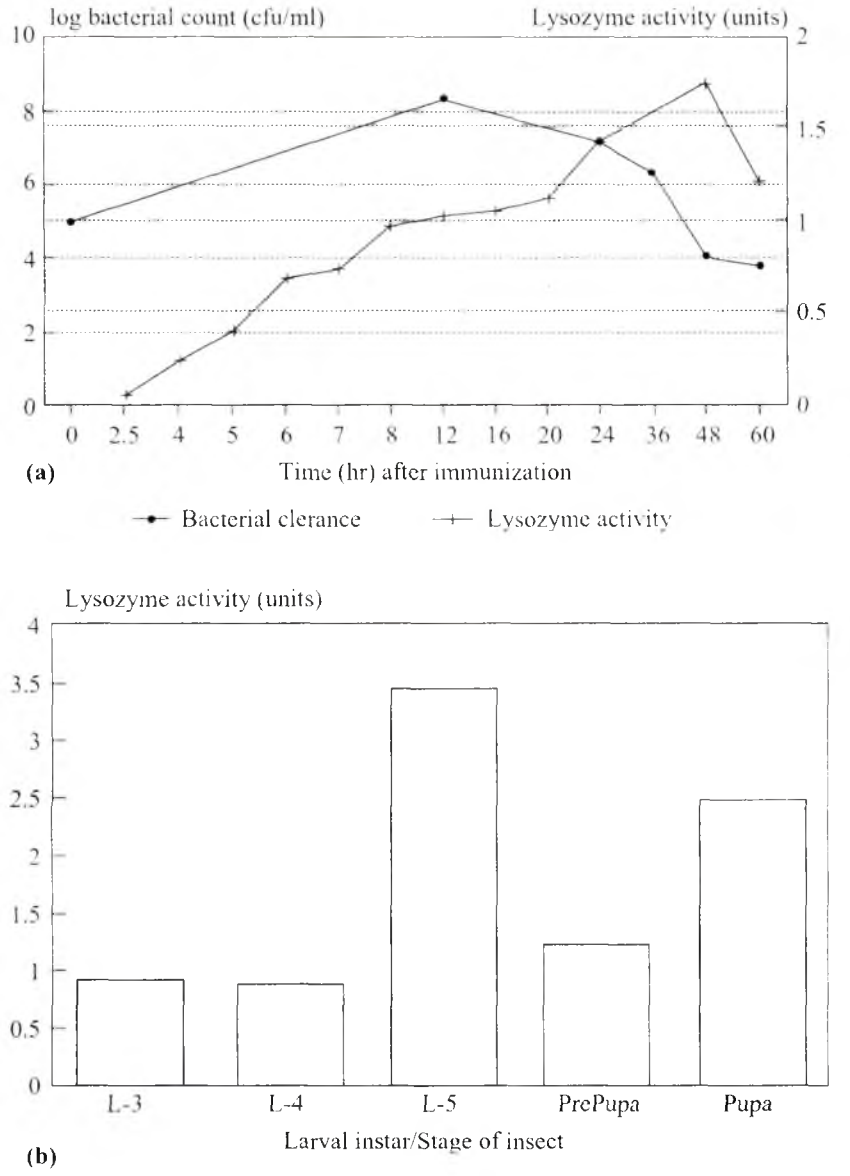

FIGURE 1. (a) Bacterial clearance and lysozyme activity in haemolymph of the last instar larva of *Helicoverpa armigera* at different time intervals on injection of larvae with *Escherichia coli* DH5α 10^6 cfu/insect. (b) lysozyme activity (units) of haemolymph of the different instars/stages of *H. armigera* immunized with *E. coli* DH5α 10^6 cfu/insect.

Lysozyme activity in haemolymph of the immune insect

Time course of induction of lysozyme activity in the haemolymph of *H. armigera* fifth instar larvae immunized with *E. coli* DH5α was studied by nephalometry. The lysozyme activity increased proportionately from 2.5 h till 8 h and then starting slowing down, after inoculation. The highest lysozyme activity, 1.74 units, was attained 48 h after inoculation of fifth instar larvae [Fig. 1(a)]. Further studies on the induction of lysozyme activity in different developmental stages showed that fifth instar larva contained the highest lysozyme activity, followed by pupa, prepupa and then younger larva, 3rd and 4th instar ones [Fig. 1(b)]. The lysozyme activity of final instar larva was 3.42, pupa 2.42, prepupa 1.17, 3rd instar larva 0.91 and 4th instar larva 0.89.

The lysozyme activity of different haemolymph samples of fifth instar larvae of *H. armigera* immunized with different bacteria and at various doses is presented in Table 1. Investigations on lysozyme activity of haemolymph by inhibition zone assay did not show any significant difference between uninjected control and saline injected ones; with both samples possessing the lowest lysozyme activity of 10.0 ng/5 μl (equivalent to chicken egg white lysozyme). The highest lysozyme activity of 5623 ng/5 μl was found in the haemolymph of larvae immunized with *E. coli* DH5α followed by *E. coli* NM522, *B. subtilis* and *E. coli*. The lysozyme activity of different immune haemolymph samples is of the order of *E. coli* DH-5α ≫ NM522 = *B. subtilis* > *E. coli* > *E. coli* DH-5>JM83. Perusal of results by nephalometric assay, however, showed that saline inoculation induced more of lysozyme activity as compared to uninjected control. Amongst bacteria, *E. coli* DH5α was the best elicitor of lysozyme induction followed by *E. coli* JM83, *E. coli* NM522 and *B. subtilis*. *B. thuringiensis* at a low dose of 10^3 cfu/insect also induced lysozyme activity in the fifth instar larvae. These studies also showed that induction of lysozyme activity depended positively upon the inoculation dose.

Antibacterial activity of immune haemolymph

The antibacterial activity of immune haemolymph of *H. armigera* was determined in terms of time of killing for different strains of test bacteria incubated with definite amount of immune haemolymph. Among the chosen bacteria, *B. megaterium* was highly sensitive to immune haemolymph of *H. armigera* with the lowest mean killing time of 13.3 min. Sensitivity of different test bacteria is of the order of *B. megaterium*≫*B. subtilis*>*E. coli* DH-5>*E. coli* NM522. *B. thuringiensis* was fully resistant to the immune haemolymph tested (Table 2).

Table 3 demonstrates antibacterial spectrum of haemolymph of larvae of *H. armigera* immunized with *E. coli* DH5α by inhibition zone assay. Among the test bacteria, *B. thuringiensis* was totally resistant to bacteriolytic action in this assay. Susceptibility of different test bacteria in this assay was of the order of *M. lysodeikticus* > *E. coli* NM522 > *B. megaterium* > other bacteria. Susceptibility of test bacteria *viz.*, *E. coli* DH5α, JM83 and *B. subtilis* was at par but slightly greater than that of *B. polymixa* and *E. coli*. In the studies on elicitation of immunity with different bacteria;

TABLE 1. Lysozyme activity in haemolymph samples of the American bollworm, *Helicoverpa armigera* immunized with different bacteria at various doses

Sl. No.	Haemolymph sample from insect treated with bacterium (injected dose)	Lysozyme activity	
		Nephalometry (units)	Inhibition zone assay (ng equivalents/5μl)
1	Control (sham injected)	0.03	10.0
2	Control (saline injected)	2.24	10.0
3	*B. subtilis* treatment (10^9 cfu/insect)	2.62	—
4	*B. subtilis* treatment (10^8 cfu/insect)	2.55	—
5	*B. subtilis* treatment (10^7 cfu/insect)	2.42	—
6	*B. subtilis* treatment (10^6 cfu/insect)	2.33	1995.26
7	*B. thuringiensis* (10^3 cfu/insect)	1.91	—
8	*E. coli* treatment (10^9 cfu/insect)	3.70	—
9	*E. coli* treatment (10^8 cfu/insect)	—	177.83
10	*E. coli* treatment (10^7 cfu/insect)	—	79.43
11	*E. coli* treatment (10^6 cfu/insect)	3.37	141.25
12	*E. coli* treatment (10^5 cfu/insect)	3.42	56.23
13	*E. coli* treatment (10^4 cfu/insect)	—	39.81
14	*E. coli* DH5α treatment (10^6 cfu/insect)	1.37	79.43
15	*E. coli* DH5α treatment (10^5 cfu/insect)	1.27	—
16	*E. coli* DH5α treatment (10^4 cfu/insect)	1.21	—
17	*E. coli* DH5α treatment (10^6 cfu/insect)	1.74	5623.41
18	*E. coli* JM83 treatment (10^6 cfu/insect)	2.74	56.23
19	*E. coli* NM522 treatment (10^6 cfu/insect)	2.62	1995.26

Insects were immunized with respective bacterium at the dose given and bled 48 h after injection.

TABLE 2. Antibacterial activity of haemolymph of last instar larvae of the American bollworm, *Helicoverpa armigera* immunized with *Escherichia coli* DH5α

Sl. No.	Test bacterium	Time of killing (min) (mean with s.e.)
1	*Bacillus megaterium*	13.33 ± 2.35
2	*Bacillus subtilis*	21.81 ± 2.35
3	*Escherichia coli* DH5α	26.67 ± 2.88
4	*Escherichia coli* NM522	31.67 ± 2.89
5	*Bacillus thuringiensis*	>1 h

Each mean is based upon 4 observations. All showed statistically significant differences at 5%. Last instar larvae were immunized with *Escherichia coli* DH5α at a dose of 10^6 cfu/insect and bled 48 h later. The immune haemolymph was tested for its antibacterial activity using test bacterium by killing assay.

TABLE 3. Antibacterial activity spectrum of haemolymph of fifth instar larvae of the American bollworm, *Helicoverpa armigera* immunized with *Escherichia coli* DH5α

Sl. No.	Test bacterium for bioassay of immune haemolymph	Mean diameter of clear zone (mm)
1	*Bacillus megaterium*	10.3
2	*Bacillus polymixa*	7.3
3	*Bacillus subtilis*	9.5
4	*Bacillus thuringiensis*	No inhibition
5	*Escherichia coli*	6.67
6	*Escherichia coli* DH5α	9.17
7	*Escherichia coli* DH5α	8.00
8	*Escherichia coli* JM83	9.67
9	*Escherichia coli* NM522	12.00
10	*Micrococcus lysodeikticus*	22.00

Last instar larvae were immunized with *Escherichia coli* DH5α at a dose of 10^6 cfu/insect and bled 48 h later. The test bacterium was spread in agar medium at the concentration of 10^6 cfu/ml of agar medium and tested for its susceptibility by inhibition zone assay.

all of the bacteria *viz*., *E. coli*, *E. coli* DH5α, *E. coli* JM83, *E. coli* NM522 and *B. subtilis* were more or less good in inducing antibacterial activity.

Acidic PAGE of immune proteins

The pooled immune haemolymph samples were analyzed by acidic PAGE at pH 4.3. Figure 2(a) and (b) show protein profiles of different immune haemolymph samples run under native conditions. Consensus banding pattern in the gel reveals that there are at least 6 different prominent proteins in the immune haemolymph samples. Protein bands marked 5 and 6 have similar mobility comparable to that of chicken egg white lysozyme. The intensity of these protein bands is also stronger as compared to control.

Figure 2(b) shows the protein profiles of different immune haemolymph samples from *H. armigera* and *A. janata* (reference sample). Comparison of the haemolymph samples from both the species reveals that there is one fast moving protein band running ahead at the cathodic side of the gel in the *A. janata* immune haemolymph sample and is not found in *H. armigera* immune haemolymph sample.

SDS-PAGE analysis of immune haemolymph proteins

SDS-PAGE analyses were carried out to estimate the molecular weight (M_r) of bacteria induced proteins in immune haemolymph [Fig. 3(a) and (b)]. As many as 30 bands could be observed in immune haemolymph samples on SDS gel and many of these bands were overlapping with that of control haemolymph samples. Based on consensus banding pattern in different immune haemolymph samples in comparison

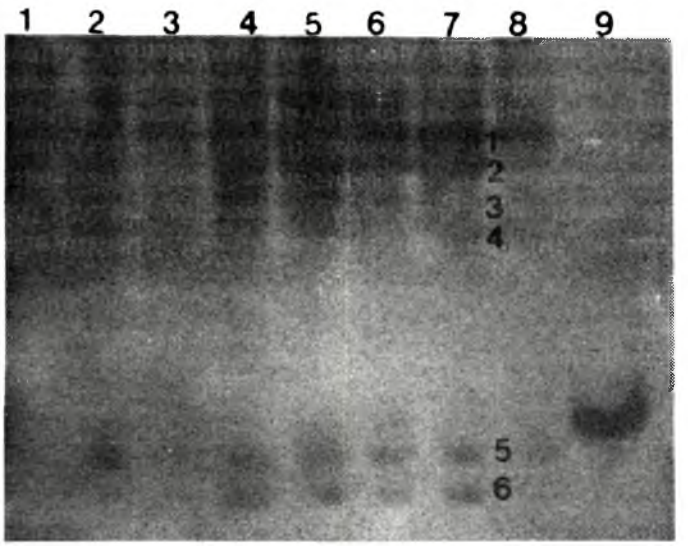

FIGURE 2. (a) Acidic polyacrylamide gel electrophoresis of haemolymph samples from immunized and normal larvae of *Helicoverpa armigera*. Lane 1-normal, 2-7 haemolymph samples from insects immunized with different *E. coli* strains and bacterium, 2-*B. subtilis*, 3-*E. coli*, 4-*E. coli* DHα, 5-*E. coli* NM522, 6-*E. coli* JM83, 7-*E. coli*, 8-normal haemolymph and 9-chicken egg white lysozyme.

with samples from control and uninjected larvae bacteria induced proteins could be deduced from the SDS gel. At least 10 major proteins were found to be bacteria induced proteins and they were designated as 'Induced Proteins' (I1-I10) (Table 4).

DISCUSSION

Clearance of bacteria from haemolymph

The clearance of bacteria from the haemolymph of the fifth instar larvae started almost immediately. Although bacterial titre showed a slight increase 12 h after inoculation, it declined with respect to time. The clearance of bacteria, which is a sure indication of activation of immune system, is also studied in lepidopterans using microbes (Ratcliffe *et al.*, 1985).

Lysozyme activity and its characterization

Concurrent with bacterial clearance, lysozyme activity increased sharply from its base level of 0.06 units 2.5 h to 0.97 units 8 h after inoculation in time dependent manner and there after attained plateau over a period of 60 h from inoculation.

Lysozyme is one of the most prominent component of humoral defense system that acts by hydrolyzing $\beta(1-4)$ linkage between N-acetyl glucosamine and N-acetyl muramic acid in the cell wall of gram positive bacteria; thereby providing protection

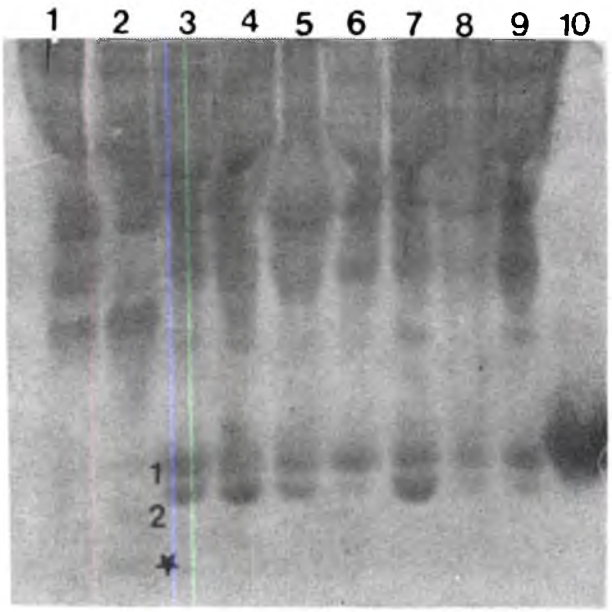

FIGURE 2. (b) Acidic polyacrylamide gel electrophoresis of different haemolymph samples, lane 1-normal haemolymph of *Achaea janata* larvae, 2-normal haemolymph of *A. janata* pupae, 3-8 haemolymph samples from insects immunized with different *E. coli* strains and bacterium [similar to Fig. 2(a)], 9-*H. armigera* normal haemolymph and 10-chicken egg white lysozyme.

TABLE 4. Major bacteria induced proteins in haemolymph of the American boll-worm, *Helicoverpa armigera*

Sl. No.	Major Bacteria induced proteins	Appr. Molecular Weight (Da)
1	I1	77,450
2	I2	49,982
3	I3a	41,886
4	I3b	39,810
5	I3c	38,018
6	I4	32,000
7	I5	28,000
8	I6	24,000
9	I7	19,600
10	I8	17,000
11	I9	16,218
12	I10	14,125

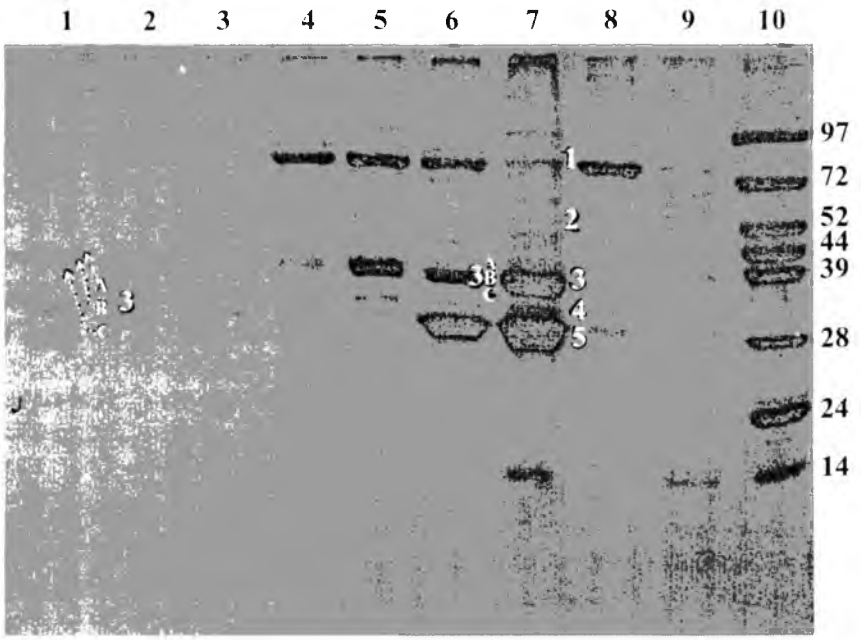

FIGURE 3. (a) SDS-polyacrylamide gel electrophoresis of different haemolymph samples of *Helicoverpa armigera* immunized with different *E. coli* strains and bacterium; lane 1-*E. coli* DH5α, 2-*B. subtilis*, 3-*E. coli* NM522, 4-*E. coli* JM83, 5-*E. coli* DH5, 6-*E. coli*,7-*E. coli* DH5α, 8-normal saline injected, 9-normal sham treated, 10- molecular weight markers.

to the host lepidopterous insects against bacterial infection. The induction of lysozyme was found to be dependent upon the bacterial species and strain and also upon dose of inoculation. Similar induction of lysozyme activity was reported in *H. armigera* by Mohrig and Messner (1968) who even observed such a response on injection of host insect with India ink and saline solution; which was in contrast to our observations. Powning and Davidson (1973) also observed a weak lysozyme activity in *Galleria mellonella* on injection with saline. Results on induction of high lysozyme activity in the host insect with bacterial inoculation were similar to those of Chadwick (1970); Anderson and Cook (1979).

Further studies on lysozyme activity in different developmental stages showed that fifth instar larvae contained more lysozyme than prepupae, pupae and young larvae. Similar results were also reported by Hughes *et al.* (1983) who suggested differential induction of lysozyme activity in various stages as a basis of physiological adaptation. Although both stages must deal with invading microbes in the haemocoel, the larvae must provide an effective defense against ingested pathogens also. These differential defense requirements might have formed the basis for additional induction of lysozyme during the actively feeding final instar larvae (Hughes *et al.*, 1983). Lysozyme activity had been detected in all stages of insect development, and has

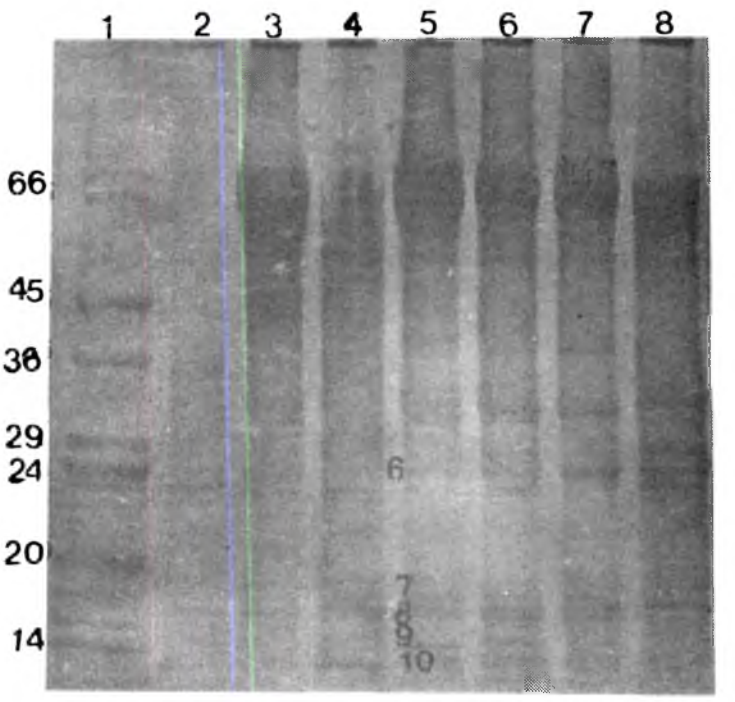

FIGURE 3. (b) SDS-polyacrylamide gel electrophoresis of different haemolymph samples. 3–8 haemolymph samples of insect immunized with different strains of bacteria.

been purified from different lepidopteran species (Powning and Davidson, 1973, 1976; Croizier and Croizier, 1980; Jollès *et al.*, 1979).

Antibacterial activity of immune haemolymph

The antibacterial activity of immune haemolymph differed with bacterial species/strains used. Of the bacteria used, *M. lysodeikticus* showed highest susceptibility. Amongst rest of other bacteria, *B. megaterium* too showed high susceptibility compared to *E. coli* strains, whereas *B. thuringiensis* was resistant. The induction of antibacterial activity did not differ with bacterium used for inoculation. These results on the antibacterial properties of immune haemolymph of *H. armigera* confirm the general characteristics of antibacterial proteins. i.e. I) Both Gram positive and Gram negative bacteria can induce non-specific immunity in *H. armigera;* II) Immune haemolymph has a broad spectrum of antibacterial activity against both Gram positive and negative bacteria.

Difference in susceptibility of test bacteria can be attributed to the molecular characteristics of antibacterial factors in the immune haemolymph. The insect antibacterial factors are fairly non-specific in nature (Boman *et al.*, 1991). Immune haemolymph from *Hyalophora cecropia* (Hultmark *et al.*, 1980), *Sarcophaga peregrina* (Okada and

Natori, 1983), *Drosophila melanogaster* (Flyg *et al.*, 1987), and *Glossina morsitans* (Kaaya *et al.*, 1987) showed a broad spectrum antibacterial action. At molecular level, this action is attributed to two main classes of antibacterial factor namely cecropins and attacins (Boman and Hultmark, 1987).

Acidic PAGE characterization

Acidic PAGE system at pH 4.3 has been a useful method for tentative identification of insect antibacterial proteins which are basic in nature. Therefore, acidic PAGE is used as the choice system for separation of antibacterial proteins of *H. armigera*. There is an evidence of inducibility of proteins as immune response in the immunized insects as indicated by the protein bands 1, 3 in Fig. 2(a) and 6, 7 (b). Although band Nos 7 and 8 can be visualised in control samples also, the relative intensity of these bands suggests that these are inducible.

Immunization with different bacterial strains did not seem to alter the immune response of insects. Irrespective of the strain of bacteria used for immunization, consensus banding pattern of different immune haemolymph samples is observed. Nonspecific induction of antibacterial proteins has been the characteristic of insect immune proteins (Boman and Hultmark, 1987).

Based on the electrophoretic mobility, there is an evidence for lysozyme like proteins in *H. armigera*, which was further corroborated by presence of as high as 500 fold levels in immune haemolymph samples of *H. armigera*. Acidic PAGE analysis shows that the mobility of two fast moving proteins is close to that of reference protein chicken egg white lysozyme. An interesting feature to be noted here is that there are two lysozyme like proteins in *H. armigera*. The presence of two lysozyme like proteins may be attributed to the presence of two forms of lysozymes in the pooled haemolymph samples. Since partially inbred laboratory population has been used in this study, we can assume existence of two alleles in the population and their expression might be giving rise to the two proteins. Alternatively, these may be two distinct proteins coded by two different genes, which might have arisen through gene duplication. Consensus electrophoretic mobility of these proteins coupled with antibacterial assays and partial purification data suggest that there are two lysozyme like proteins in *H. armigera*.

Lysozymes are purified and characterized in haemolymph of *Heliothis virescens* (Lockey and Ourth, 1996). Multiple lysozymes have earlier been detected in duck egg white (Jollès *et al.*, 1979) and cricket *Gryllus bimaculatus* (Schneider, 1985). Analysis of duck egg white lysozymes reveals that they are due to multiple alleles at a single locus (Prager and Wilson, 1971). Studies on cricket lysozymes considered the possibility of existence of a single locus with multiple alleles or duplicated gene loci for the two forms of the enzyme.

An intriguing aspect of *H. armigera* immune response is that cecropin-like factors could not be detected during studies by acidic PAGE. Whereas a low molecular weight peptide akin to cecropin moving ahead of chicken egg white lysozyme could be detected in immune haemolymph of *A. janata* pupae (reference sample) [Fig. 2(b)].

Close scrutiny of the data on antibacterial spectrum of immune haemolymph of *H. armigera* showed bacteriolytic action on both Gram positive and negative bacteria (Table 3). Lysozymes are bactericidal only against some but not all Gram positive bacteria. However cecropins can also act on those bacteria susceptible to lysozymes (Boman *et al.*, 1991).

Cecropins are the major antibacterial factors found to be induced upon immunization of several lepidopterans (Hoffmann *et al.*, 1981). Comparative analysis of amino acid sequences also shows that cecropins from different lepidopterans share a homology (Boman and Hultmark, 1987). Cecropins constitute only a few per cent of total antibacterial proteins in insects (Boman *et al.*, 1991). Perhaps due to low level, cecropins could not be detected in the acidic PAGE analysis of immune haemolymph samples. Another possibility is that a significant part of the antibacterial action in the haemolymph might have lost by proteolytic degradation although phenyl methyl sulfonyl fluoride and ethylene diamine tetraacetic acid were added while collecting the immune haemolymph samples. In *Drosophila*, cecropins could not be detected in the haemolymph due to proteolytic degradation (Flyg *et al.*, 1987). Circumstantial evidences thus suggest presence of cecropin-like factor in *H. armigera*.

SDS-PAGE analysis of immune haemolymph proteins

Among the different bacteria induced proteins I1, I3, I5, I9 and I10 were induced to higher levels than other proteins. Some of these proteins like I1, I3, I9 and I10 were induced to a lesser extent in saline injected larvae also. Faint bands of I1, I3 and I9 could be observed in uninjected control samples also. Three forms of I3 could be visualized in the gel. I5 seemed to be a family of proteins with approximate M_r of 28 kDa. Bacteria inducible proteins were designated as I1-I10 based on two criteria: (i) Relative intensity of the protein bands in the immune and control haemolymph samples (I1, I3, I5, I9), (ii) Additional bands that could be visualized in immune haemolymph samples than in uninjected control ones (I2, I4, I6). By comparative analysis of approximate molecular weights with that of earlier workers (Faye *et al.*, 1975; Hughes *et al.*, 1983; Hulbert *et al.*, 1985), we can identify the bacteria induced proteins of *H. armigera*.

1. I1 group of proteins corresponds to P2 of *H. cecropia* having the approximate M_r of 77 kDa.
2. I2 may correspond to Haemolins of *H. cecropia* and other lepidopterans with approximate molecular weight of 50 kDa.
3. High induction of I3 group of proteins is observed. I3 seems to exist in 3 forms with approximate M_r each of 41 kDa, 39 kDa and 38 kDa respectively. Relative intensity of three forms of I3 group in *H. armigera* indicates that two forms, B and C may be major inducible proteins in larvae. No antibacterial activity has been associated with any of these proteins so far. These proteins may correspond to M13 group of proteins in *Manduca sexta* (Hulbert *et al.*, 1985). It was reported that these groups of proteins were differentially induced in larvae and pupae. Of

the three forms of M13 group A and C were major inducible proteins in larvae and B was induced in pupae.

4. I4 is induced in bacteria injected as well as saline injected ones. Approximate M_r is 32 kDa. Function of this protein is not known.
5. I5 is the highly inducible proteins among the inducible proteins of *H. armigera* with the approximate M_r of 28 kDa [Fig. 3(a)].
6. I6 is a group of proteins having approximate M_r of 24 kDa. This group may represent attacins of *H. cecropia* and other lepidopterans. A group of six attacins have been described in *H. cecropia* (Hultmark *et al.*, 1983).
7. I7 group of proteins has an average M_r of 19.6 kDa. This may belong to M16 of *M. sexta*. The functional significance of this protein is not known.
8. I8 group of proteins has average M_r of 17 kDa. I8 is seen in control as well as saline injected samples as faint bands. This may represent the lysozymes of lepidopterans (Boman and Hultmark, 1987). Inducible activity of lysozymes has been reported in Lepidoptera (Chadwick, 1970; Powning and Davidson, 1973).
9. I9 group of proteins has approximate M_r of 16 kDa. In *M. sexta*, a group of 16 kDa proteins was found to be induced in pupae but not in larvae.
10. I10 group has an average M_r of 14 kDa. This band could be visualized in control and saline injected larvae also. Inducibility of these proteins could be visualized distinctly in immune haemolymph samples. This could also be a lysozyme like protein. The present investigations suggest that there may be two lysozyme like proteins and I10 group may probably represent lysozyme-II.

Thus, the present study reveals that there are at least 10 major proteins induced by bacterial injections to the larvae of *H. armigera*. By selective labelling of diapausing pupae of *H. cecropia*, Faye *et al.* (1975) could observe the induction of 8 different proteins in immunized insects. Hughes *et al.* (1983) and Hulbert *et al.* (1985) reported as many as 18 major bacteria inducible proteins in the haemolymph from larvae of *M. sexta*. Abraham *et al.* (1995) also reported purification and characterization of antibacterial proteins in *Bombyx mori*.

Partial purification of immune haemolymph proteins

Partial purification of two lysozyme like proteins from the remaining haemolymph proteins was attempted by initially fractionating on Sephadex G-100 to reduce the amount of competing proteins. Individually all the fractions were analyzed for antibacterial activity against *M. lysodeikticus* and by acidic PAGE. Antibacterial activity spread widely over fractions 10 through 29 in two pools I & II. Pool I fractions analysis were shown to contain both the lysozyme-like proteins and pool II fractions contained only lysozyme like protein-I only. Pooled fractions were passed through CM Sepharose at pH 5.0. Chromatogram illustrates that antibacterial fractions were distributed in fractions 30 through 37. These fractions contained a major protein corresponding to lysozyme like protein-I based on electrophoretic mobility. Pooled fractions when rechromatogramed again with CM Sepharose a single peak was

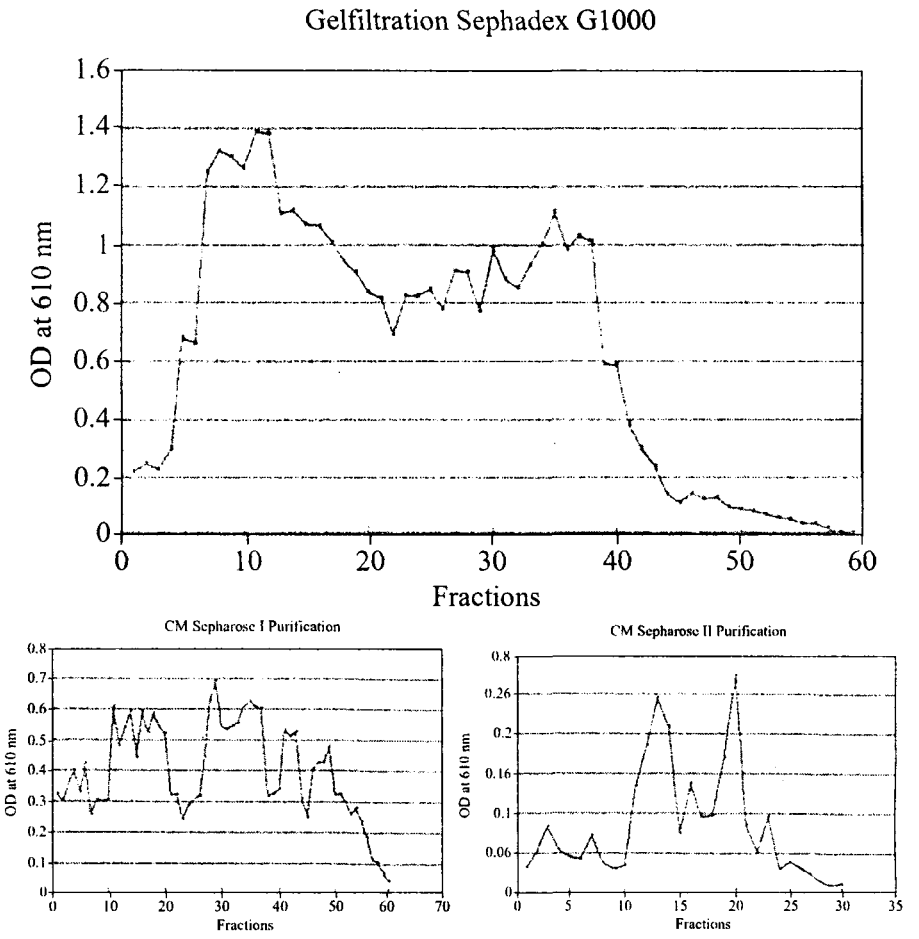

FIGURE 4. Partial purification of immune haemolymph proteins of the American bollworm, *Helicoverpa armigera*.

obtained (peak No. 10). This fraction had well anti-*M. lysodeikticus* activity and had same eletrophoretic mobility as that of lysozyme like protein I. This peak also showed a minor contaminant in acidic PAGE. Second lysozyme like protein could not be retained in the CM Sepharose column at pH 5.0. It is quite likely that the basicity of this factor is strikingly different to that of lysozyme like protein I. It is evident from the present study that antibacterial activity of these proteins possesses a physiological role in the insect immunity (Kaaya, 1989; Boman, 1995; Hetru *et al.*, 1998).

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Mechano-and Chemoreceptors and their Possible Role in Host Location Behaviour of Parasitoid *Anisopteromalus calandrae* Howard (Hymenoptera : Pteromalidae)

S. Ignacimuthu*[1] and S. Dorn[2]

[1]*Entomology Research Institute, Loyola College, Chennai 600034, India*
[2]*Institute of Plant Sciences, Applied Entomology, ETH, Clausiusstr 25, CH-8092 Zurich, Switzerland*
Email: eri_lc@hotmail.com

ABSTRACT: Electron microscopic studies of the sensilla morphology of the antennae and tarsus of parasitoid *Anisopteromalus calandrae* revealed a wide range of types which act as mechano-and chemoreceptors. The antennae of the female parasitoid had 4 types of sensilla. Several types of trichoid sensilla were found at the antennal tip. The tarsomeres were covered by three types of mechanoreceptors. At the pretarsus, sensilla were found on the manubrium and claws. The claws had contact chemoreceptors and mechanoreceptors, and the manubrium had only mechanoreceptors, a trichoid sensillum and companiform sensilla. The sensilla inventory was compared with other parasitoid species. The role of different sensilla is discussed with respect to their functional aspects.

KEYWORDS: Mechano-and Chemoreceptors, *Anisopteromalus calandrae*, parasitoid, sensilla, tarsomeres.

INTRODUCTION

There is a strong contrast between the large number of investigations done on parasitoid searching behaviour (Godfrey, 1994) and studies on morphology of parasitoid sensilla. Most studies focus on the antenna, because many chemoreceptive sensilla are located there and because the antennal tapping behaviour of foraging parasitoid is striking. In contrast, studies on tarsal sensilla are extremely scarce. All types of sensilla including tarsal sensilla of the parasitoid *Trichogramma minutum* has been studied (Schmidt and Smith, 1987). They recorded many sensillae, of unknown function on the tarsi. *Anisopteromalus calandrae* is an ectoparasitoid of the larvae of *Callosobruchus* spp. Electron microscopy was used to identify sensilla on the extremities' of *A. calandrae* that might play a role in the perception of various signals and cues. Such

*Corresponding author

sensilla are most likely to be found on the tarsi and antennae, because these body parts come into direct contact with the seed surface. This study was carried out with a view to study the structure and variation of different kinds of sensilla.

MATERIALS AND METHODS

The terminology and nomenclature of Altner and Prillinger (1980) was used for the different types of cuticular sensilla. Where necessary references were made to older nomenclature (Schneider, 1964). Female parasitoid was anaesthetized using CO_2 and prepared by a standard chemical fixation method. Prefixation in the standard chemical fixation was done for 12 h at 4 °C with 5% glutaraldehyle, 5% formaldehyde, in 0.2 M sodium cacodylate buffer pH 7.4. After washing with buffer the specimens were immersed in a postfixative of 2% osmium tetroxide in 0.2 M sodium cacodylate buffer (pH 7.4) for 2 h at 4 °C. Females were then dehydrated in a graded ethanol series, critical point dried in a Balzers Union CPD 030 unit, and mounted on a scanning electron microscope stub with double-sided sticky tape. Preparations were sputter coated in Balzers Union SCD 040 with gold-palladium for 3 min. Specimens were viewed in a Hitachi S 700 scanning electron microscope at an accelerating voltage of 15 KV. Images were recorded digitally on a computer with the Digiscan interface (GATAN), and then arranged with the image processing software Adobe Photoshop (Adobe Systems, 1996).

RESULTS

The antenna of a female parasitoid consists of a basal scape, a pedicel and a flagellum formed by 9 annuli [Fig. 1(a)]. The scapus and pedicel had only few sensilla and the surface was shaped in a pattern of overlapping scales. The first 7 of the 9 annuli were of almost equal length and the last two at the tip of the flagellum were smaller. These two may form a functional unit because they were closer to each other than the first 7.

On each of the 9 annuli of the flagellum, there were 4 different types of sensilla. There were distributed equally on the surface of the annuli. All hair shaped sensilla pointed distally, with their longitudinal axis parallel to the longitudinal axis of the antenna. Two of the four different sensilla types located on the antenna were trichoid sensilla. Type A1 [Fig. 1(b1)] was socketed hair-shaped sensilla and was spread over the whole surface. Type A2 [Fig. 1(b2)] was a round hair shaft without socket. Both trichoid sensilla had neither wall pores nor a terminal pore. The third type A3 multiporous plate sensillum *(Sensillum plecadeum)*, was also hair-shaped and unsocketed, but had wall pores [Fig. 1(1d)]. The fourth type of sensillum is called peg-like sensillum *(Sensillum coeloconicum)*. This sensillum originated in a depression [Fig. 1(1e)] and was classified as A4.

The tip of the antenna had a high density of sensilla. It had an oval shaped protuberance [Fig. 1(c)]. Two different socketed trichoid sensilla originated from this structure.

Each tarsus consisted of 4 tarsomeres and the pretarsus [Fig. 2(a)]. Tibia revealed

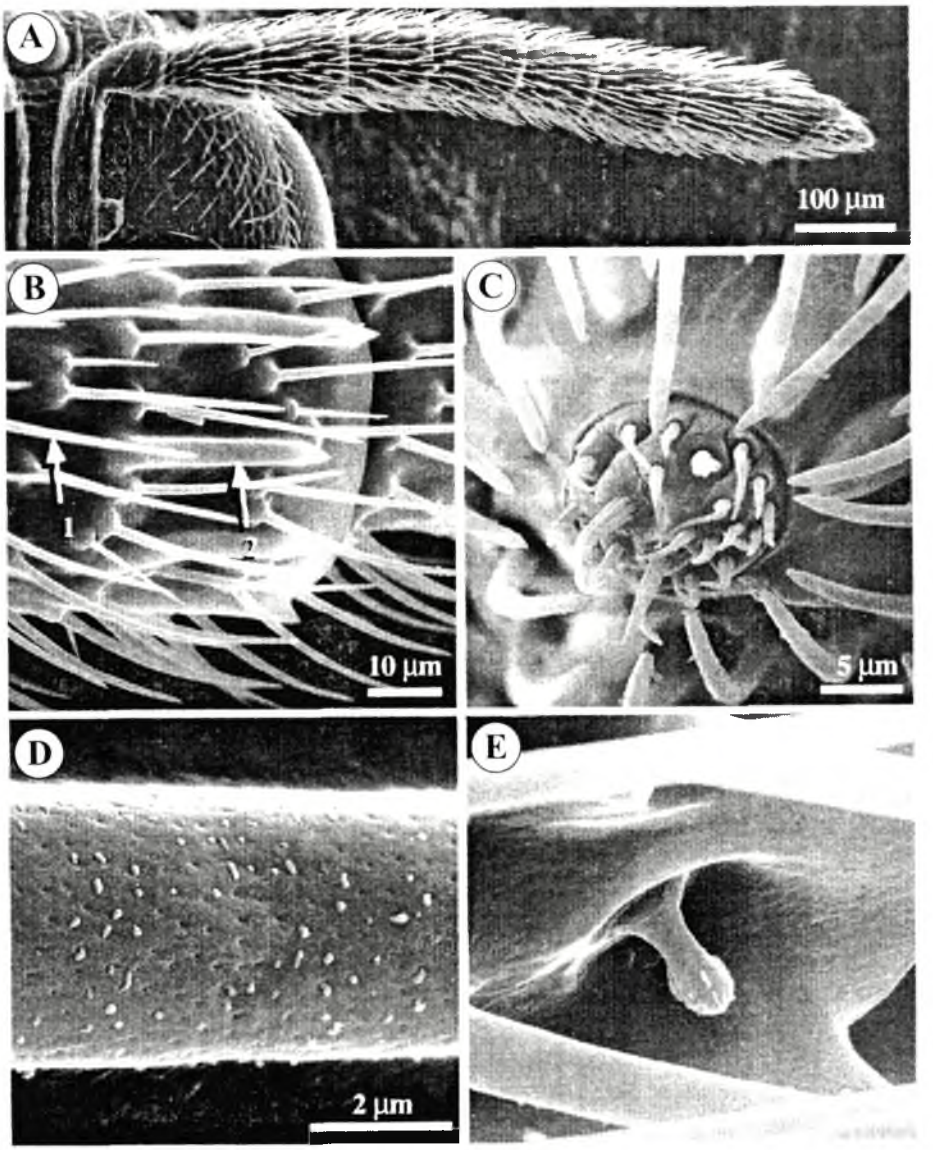

FIGURE 1. (A) Antennae of *A. calendrae* with 9 annuli on the flagellum, the scape and the pedicel; (B1) Socketed hair-shaped sensilla (Type-A1) on the surface of the annuli; (B2) Unsocketed microtricha (Type-A2) on the surface of the annuli. (C) Tip of the antenna with oval shaped protuberance. (D) Multiporous plate sensillum with wall pores (Type-A3). (E) Peg-like sensillum (Type-A4).

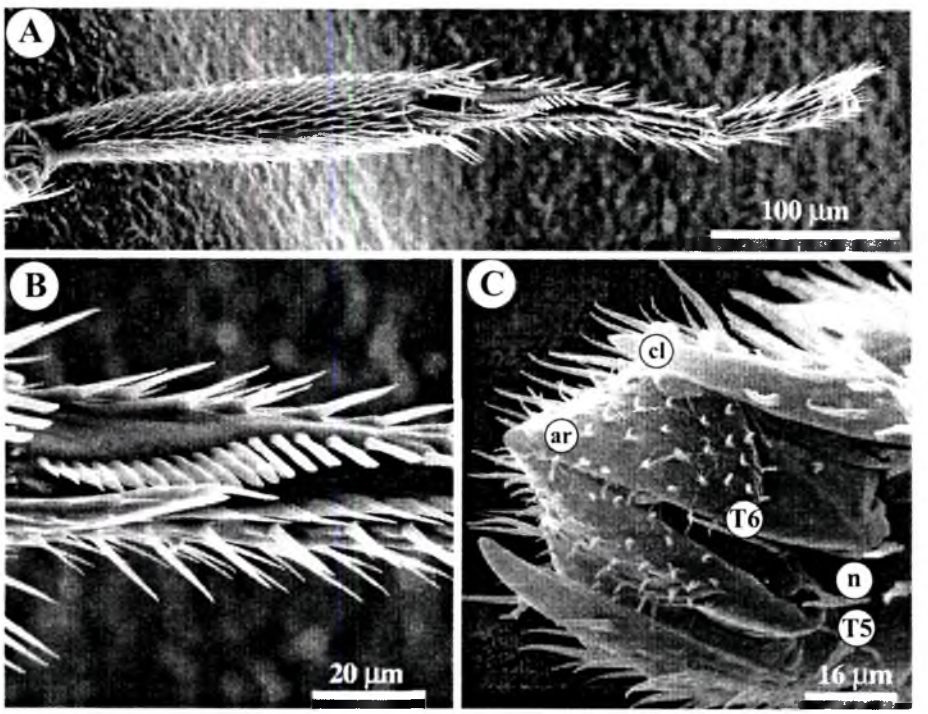

FIGURE 2. (A) Tarsus with 4 tarsomeres and the pretarsus. (B) Trichoid sensilla on the tibia. (C) Pretarsus; n - cuticular manubrium; ar - membranous arolium; cl - claw; T5 - unsocketed microtricha on the manubrium; T6 - prominent socketed hair.

a different inventory of trichoid sensilla than the tarsomeres [Fig. 2(b)]. The pretarsus was divided into a cuticular manubrium (Fig. 2(c), n), a membranous arolium (Fig. 2(c), ar), and the claws (Fig. 2(d), cl). The same sensilla types were found on all 6 tarsi. The surfaces of the tibia and femur are sculptured in a pattern of overlapping scales. Only the tibia had a dense arrangement of socketed hairs [Fig. 2(a)] whereas the surface of the femur was only sparsely covered by hairs. On the manubrium, a group of 5 unsocketed microtricha was arranged in a horizontal row (Fig. 2(c), Type T5) perpendicular to the long axis of the tarsomeres. A particular prominent socketed hair was found distal of the group (Fig 2(c), Type T6). The tip of the prominent hair did not overlap the pretarsus. The claws had 3 different types of hairs. Laterally, unsocketed microtricha of Type-T1 were found. Distal on the ventral side of the claw a hair with a dicondylie articulation was found. This hair was specifically affected in its position to the substrate by the evagination process of the arolium. Another hair found ventro-lateral on the claw had a wrinkled hair shaft and was socketed. The socket structure with its cuticular flanges restricted the movements of the hair.

DISCUSSION

Two studies on the morphology of the external sensilla that also analysed parasitoid legs was done (Schmidt and Smith, 1987; Meyhofer *et al.*, 1997). They describe the general morphology of the parasitoid *Trichogramma minutum* and *Sympiesis sericeicornis*, respectively. The external morphology of the *Trichogramma* and *Sympiesis* tarsus and pretarsus seems to be quite similar to that of *A. calandrae* as is common for most Hymenoptera (Snodgrass, 1956). Invagination ability of arolium was reported in *Sympiese* (Meyhofer *et al.*, 1997). Another unique type of sensillum are the campaniform sensilla located on the manubrium. External components of companiform sensilla generally appear as round or oval domes on the cuticle surface surrounded by a raised rim or socket (Grunert and Gnatzy, 1987).

Four different types of sensilla located on the pretarsus of *A. calandrae* have mechano-receptive properties: trichoid sensilla on the claws and on the manubrium and companiform sensilla located at manubrium. Based on the observations of Gnatzy and Schmidt (1971). Dumpert and Gnatzy (1977) and Meyhofer *et al.* (1997) describe a similar spatial arrangement of companiform and cercal filiform hairs. We suggest the following proposals; (1) The tip of the trichoid sensillum on the manubrium is in contact with the evaginated arolium and perceives cibrational signals transimitted through the membranous arolium, which (2) filters vibrational signals. (3) Associated with the trichoid sensillum are 3 companiform sensilla, which monitor extreme deflections of the trichoide sensillum causing stress in the cuticle. (4) Finally, the companiform sensilla monitor the evagination/invagination process itself.

The evagination process of the manubrium also affects the position of the innervated trichoid sensilla located on the claws; different angles between the hair shaft and the substrate suggest that this process alter the stimulus reaching the neurone. The chemoreceptive sensilla on the antennae of parasitoids are described as multipurous plate sensilla (MPS) (Barlin and Vinson, 1981). In chalcidoidea the MPS exist in two different forms. The main morphological difference between the two forms is the thickness of the cuticular wall. The external morphology of the MPS on the antennae of *A. calandrae* was similar to that of other chalcidoidea. The numerous wall pores clearly indicate that they function as olfactory chemoreceptors.

Functions of antennal sensilla other than the MPS are not clearly known. The peg-like sensillum (sensillum coeloconieum) found in *A. calandrae* was also found on the antennae of *Nasonia vitripennis, Perdesia discus* (Pteromalidae) (Miller, 1972), *Trybliographa rapae* (Cynipidae) (Butterfield and Anderson, 1994) and *Sympiesis sericeicornis* (Gnatzy and Schmidt, 1971). These species represent a broad range of parasitoid families. Coeloconic sensilla with the same external morphology have been described as thermohygroreceptors for other insects (Altner and Prillinger, 1980).

Our morphological study of the sensilla of *A. calandrae* reveals that antennal sensilla have mostly chemoreceptive properties and tarsal sensilla have mostly mechanoreceptive properties and could be involved in reception of vibrational signals.

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Effect of Plumbagin and Azadirachtin on Cuticular Proteins of *Helicoverpa armigera* (Hübner) (Lepidoptera : Noctuidae)

A. Josephrajkumar[1] and B. Subrahmanyam*[2]

[1]*Cardamom Research Station, Pampadumpara 685556, Kerala, India*
Email: bsub_ent@iari.ernet.in
[2]*Division of Entomology, Indian Agricultural Research Institute, New Delhi 110012, India*

ABSTRACT: Analysis of cuticular proteins by SDS-PAGE (15%) was carried out to ascertain the effect of two insect growth regulators *viz.*, plumbagin and azadirachtin on cuticulogenesis at larval-pupal transformation. Urea soluble cuticular protein content of the control insects reached a maximum at the late pre-pupal stage and the treatments reduced the protein content significantly. The cuticular protein profile of the treated insects showed variation in the number and prominence of bands compared to that of control insects. In the early pre-pupal stage, most of the prominent bands were in the range of 39.2–15.5 kD. In plumbagin-treated insects, four extra bands of 41.0, 23.4, 14.7 and 13.8 kD were stained appreciably whereas in azadirachtin-treated insects, a total of only five bands of 32.7, 18.8, 17.6, 15.5 and 13.8 kD were intensely stained. These changes suggest defective chitin-protein cross linking and sclerotization of pupae.

KEYWORDS: Plumbagin, Azadirachtin, Cuticular proteins, *Helicoverpa armigera*.

INTRODUCTION

Proteins form an integral part of the cuticle and play an important role in melanization and sclerotization. While a great majority of proteins are basic, the hydrophilic proteins present in larval cuticle of Lepidoptera have only relatively weak interactions, permitting the stretching and expansion during larval maturation (Andersen *et al.*, 1986, 1995). There has been increasing evidence on the effect of insect growth regulators (IGR) on cuticular protein synthesis. Juvenile hormone and its analogs alter the protein content and retain cuticle's characteristic juvenile protein profile while compounds like MON-0585 cause post-translational modifications (Chen and Mayer, 1985). Feeding tebufenozide, an ecdysteroid agonist, to final instar of the spruce bud worm, *Choristoneura fumiferana* leads to failure of expression of cuticular protein LCP 14 and the tanning enzyme dopa decarboxylase in the new cuticle that forms

*Corresponding author

precociously (Retnakaran *et al.*, 1997). Lepidopterans treated with azadirachtin and plumbagin show disturbance in cuticulogenesis, visible externally as unsclerotised or incompletely tanned areas in pupal state (Mordue and Blackwell, 1993; Krishnayya and Rao, 1995). The present investigation was therefore carried out to determine the effect of these two IGR compounds on the cuticular proteins of the gram pod borer, *Helicoverpa armigera* during larval-pupal transformation.

MATERIALS AND METHODS

Insect culture

H. armigera was maintained on a semisynthetic diet (Singh and Rembold, 1992) in an insectary at 25 ± 1 °C, 14L : 10D photoperiod and 65% R.H. Plumbagin was topically applied at the ED_{50} dose (Krishnayya and Rao, 1995) of 100 μg/g body weight in 2 μl of acetone, 0–4 h after moulting into the final-instar. Azadirachtin (dissolved in 50% ethanol in 0.7% NaCl) was injected in 2.5 μl volume of the solvent into one-day old final-instar larvae at the ED_{50} dose (Mordue and Blackwell, 1993) of 1.25 μg/g body weight. Injection of azadirachtin was preferred due to its strong oral antifeedant and low contact activity. Plumbagin was obtained from Aldrich Chemical Company, USA and azadirachtin (99% purity) was a gift from Professor Rembold, Max Planck Institute for Biochemistry, Munich, Germany.

Protein estimation

Dorsal thoracic cuticles from plumbagin-treated, azadirachtin-treated and control larvae were dissected out at various intervals, cleaned rapidly and carefully by scraping with a fine scalpel in 1% sodium tetraborate. Cuticles from two or three insects constituted one replication and five such replications were taken for each stage. The pieces of cuticle were washed briefly in distilled water and the excess moisture was absorbed on a filter paper. Cuticles of known weight were extracted overnight in 200 μl of 6 M urea in 0.1% trifluoroacetic acid at 4 °C, without homogenization (Andersen *et al.*, 1995). An aliquot was used for quantitative determination of protein using bovine serum albumin as standard (Lowry *et al.*, 1951).

Electrophoresis

Proteins were separated by SDS-PAGE according to Laemmli (1970) using vertical slab gel unit (LKB-2001). Samples of 100 μg protein were mixed with equal volume of sample buffer without β-mercaptoethanol, incubated overnight at room temperature and loaded on 15% polyacrylamide gel. Electrophoresis was run at 14 °C and at a constant current of 10 mA per sample well. Low molecular weight protein markers were also loaded along with the samples. The gels were stained with Coomassie brilliant blue and scanned on a laser densitometer (LKB). The absorbance values of protein fractions were plotted against their respective relative mobility (R_m) values using a uniform scale and presented graphically. Molecular weights of all the protein bands in the sample were interpolated using a regression equation drawn between R_m values versus log-molecular weight of the standard protein markers.

TABLE 1. Protein content of the thoracic cuticle of last instar *H. armigera*

Age and physiological stage of control	Protein content of fresh cuticle (mg g^{-1})			CD ($P = 0.05$)
	Control	Plumbagin (ED_{50})	Azadirachtin (ED_{50})	
40 h (Active feeding)	$157.75^{b} \pm 1.79$	$248.22^{a} \pm 7.56$	$117.71^{c} \pm 1.37$	7.29
80 h (Wandering)	$85.93^{b} \pm 2.12$	$93.44^{b} \pm 6.91$	$103.62^{a} \pm 4.54$	7.88
120 h (Early pre-pupal)	$103.73^{b} \pm 5.48$	$112.53^{a} \pm 4.01$	$97.54^{b} \pm 1.98$	6.53
150 h (Late pre-pupal)	$175.16^{a} \pm 6.87$	$150.21^{b} \pm 7.58$	$119.83^{c} \pm 12.36$	14.82

Values represent the mean (±SE) of four replications.
Row means followed by the same letter are not significantly different.

Statistical analysis

The means of cuticular proteins content at the four stages were subjected to analysis of variance and significance of the differences among the means was determined by F-test comparing the two treatments and control (Gomez and Gomez, 1984).

RESULTS

Protein content

Data on the protein concentration of dorsal thoracic cuticle, expressed as mg/g fresh cuticle, are presented in Table 1. In control insects, protein content was higher (158 mg/g) during the active feeding stage (40 h), dropped significantly at wandering stage and increased steadily and reached in a maximum of 175 mg/g at the late pre-pupal stage (150 h). Protein content of plumbagin-treated insects was significantly higher (248 mg/g) at the active feeding stage than in control. However, it was found to be significantly lower (150 mg/g) than in control at the late pre-pupal stage. In azadirachtin-treated larvae, the protein content was significantly higher (104 mg/g) at 80 h while at other three stages of analyses, it was found to be significantly lower than in control. Variation in the cuticular protein content at the four different stages was very narrow (98–120 mg/g) in case of azadirachtin injected larvae. It varied within a wide range in control (86–175 mg/g) as well as plumbagin-treated insects (93–248 mg/g).

Protein profile

The electrophoretic patterns of proteins of the control, plumbagin and azadirachtin-treated insects are presented in Plate 1. The cuticular protein profile of the treated insects, as observed from the electrophorograms and densitometric scanning (Fig. 1) showed variation in the number and prominence of bands compared to those of control insects. A maximum of 21 bands, whose intensity exceeded 0.25 absorbance units (AU), could be detected. Not all proteins were present at all stages of development and certain bands altered in their relative intensities. Most of the protein bands are in the medium to low molecular weight range of 41.0–11.7 kD (Table 2).

TABLE 2. Profile of the prominent cuticular proteins of last instar *H. armigera*

Sl. No.	R_m value	Mol. wt kD*	Control (h)				Plumbagin (h)			Azadirachtin (h)			
			40	80	120	150	80	120	150	40	80	120	150
1	0.209	41.0	–	–	–	–	–	+	–	–	–	–	–
2	0.225	39.2	–	–	+	–	–	+	–	–	–	–	+
3	0.270	34.6	–	–	+	+	+	+	+	–	–	–	+
4	0.290	32.7	–	+	+	+	+	+	+	–	+	+	–
5	0.322	29.9	–	+	+	+	+	+	+	–	–	–	–
6	0.340	28.5	–	+	+	–	–	+	+	–	–	–	–
7	0.382	25.3	–	–	+	–	+	+	+	–	–	–	+
8	0.400	24.1	–	–	+	–	+	+	+	–	–	–	–
9	0.411	23.4	–	–	–	+	+	+	+	–	–	–	+
10	0.424	22.5	–	+	+	+	+	+	+	–	–	–	–
11	0.444	21.3	–	+	+	+	+	+	+	–	–	–	–
12	0.464	20.2	–	+	+	+	+	+	+	–	+	–	+
13	0.490	18.8	–	+	+	–	+	+	+	–	+	+	–
14	0.500	18.3	–	+	+	–	+	+	+	–	+	–	–
15	0.514	17.6	–	+	+	–	+	+	+	–	+	+	+
16	0.538	16.4	–	–	+	–	+	+	+	–	–	–	+
17	0.560	15.5	–	–	+	–	+	+	+	–	+	+	+
18	0.579	14.7	–	–	–	–	+	+	+	–	+	–	+
19	0.600	13.8	–	–	–	–	+	+	+	–	–	+	+
20	0.624	12.9	–	–	+	–	+	+	–	–	+	–	–
21	0.660	11.7	–	–	+	–	–	–	–	–	+	–	–

* Intensity of bands above 0.25 absorbance units.

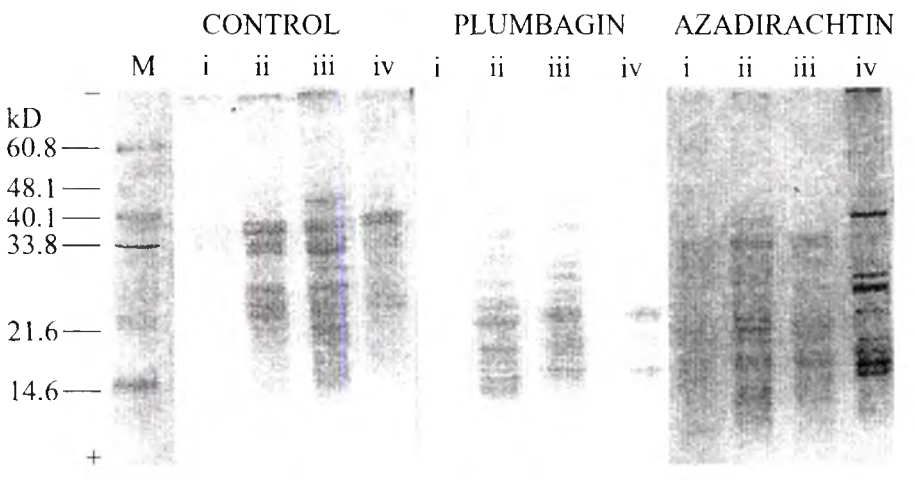

Plate 1 Electrophoretic profile of cuticular proteins of last instar *H. armigera* on SDS-PAGE (15%) M: Molecular weight marker proteins i, ii, iii and iv: 40, 80, 120 and 150 h old larvae.

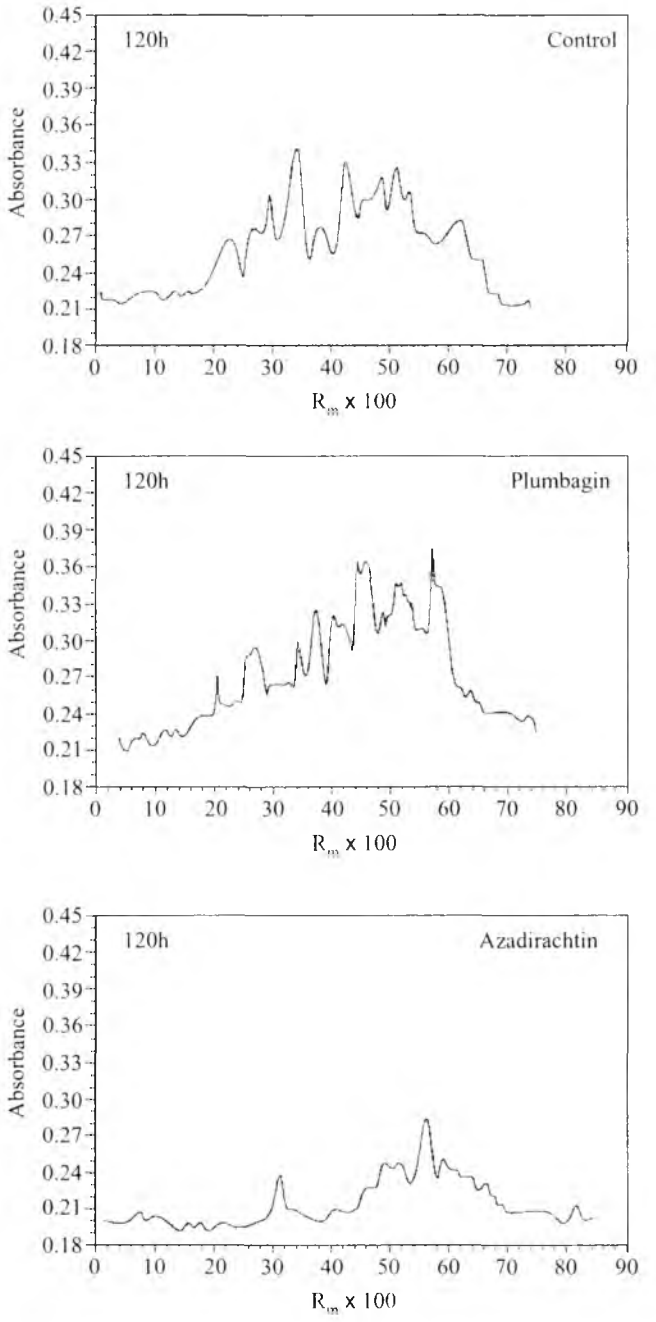

FIGURE 1. Densitograms of cuticular proteins of last instar *H. armigera*

The intensity of staining of proteins at the active feeding stage of control was low, increased at wandering and early pre-pupal stage and reduced further at late pre-pupal stage. The number of prominent bands at the above mentioned stages were 0, 9, 17 and 7, respectively. Despite higher protein content at the active feeding stage and late pre-pupal stage, the intensity of staining of proteins was very low. From the protein profile of plumbagin-treated insects, it is evident that no prominent band could be observed at 40 h. As the growth progressed, the intensity of staining increased without major differences in the profile at each stage. The total number of prominent bands were 0, 17, 20 and 17, respectively, at the four stages. The protein profile of azadirachtin-treated insects did not show any intensely stained band at 40 and 120 h. However, the intensity of staining was well pronounced at 80 and 150 h. The total number of prominent bands were 0, 9, 5 and 10 at 40, 80, 120 and 150 h, respectively. In control, medium molecular weight proteins (39.2–25.3 kD) were intensely stained whereas in both the treatments, only low molecular weight proteins (22.5–13.8 kD) were stained appreciably.

Based on the prominence of a majority of proteins at 120 h in both the treatments and control (early pre-pupal stage), a direct comparison was made at this stage. In the control group, most of the prominent bands were located in the molecular weight range of 39.2–15.5 kD. In plumbagin-treated insects, four extra bands of 41.0, 23.4, 14.7 and 13.8 kD were stained appreciably, whereas in azadirachtin-treated insects a total of only five bands of 32.7, 18.8, 17.6, 15.5 and 13.8 kD were intensely stained. The latter was devoid of prominent bands in the range of 41.0–34.6 kD and 29.9–20.2 kD.

DISCUSSION

Urea soluble protein content of *H. armigera* larval cuticle in this study ranged from 8.5–17.5% of fresh cuticle. Krishnayya and Rao (1995) earlier reported 23.68–35.25% protein in freeze dried cuticles of the same insect, extracted into 6 M urea, following homogenization. The protein content was higher during the active feeding stage and later it dropped significantly suggesting epidermal reprogramming from larval to pupal cuticle. In the control group, high protein content was observed in late pre-pupal stage, when pupal transformation was nearing completion. In male fifth-instar migratory locust *Locustsa migratoria* also, the total soluble cuticular proteins increase during the first two days prior to adult ecdysis (Phillips and Loughton, 1975).

In plumbagin-treated insects, the trend was similar but the protein content was very high initially at the active feeding stage and could not attain maximum unlike in the control group before pupation. In azadirachtin-treated insects, though protein content was the highest at late pre-pupal stage, it was still lower than that of plumbagin-treated insects. Epidermal reprogramming and synthesis of new cuticular proteins at metamorphic moult are regulated by the moulting hormone (Andersen *et al.*, 1995). In fact, plumbagin and azadirachtin at ED_{50} doses drastically deplete the critical ecdysteroid peaks of *H. armigera*, leading to defective metamorphic moult (Josephrajkumar *et al.*, 1999).

Cuticular proteins of *H. armigera* noticed in this study exhibited qualitative differences in electrophoretic profiles. The intensity of bands varied at each of the stages analysed. Though the protein content was very high at the active feeding stage, none of the bands was stained appreciably beyond 0.25 AU suggesting that they are basic proteins which could be separated by cationic PAGE and also by isoelectro focussing. It is noteworthy that the last larval instar of *H. armigera* undergoes a behavioural change coinciding with the appearance of specific and new cuticular proteins that are regulated by moulting hormone.

The second critical ecdysteroid peak of *H. armigera* noticed in our earlier study (Josephrajkumar *et al.*, 1999) initiates epidermal apolysis, degradation of larval cuticle and the synthesis of pupal cuticle with proteins that are basic. Similar changes in ecdysteroid titre have also been reported to occur prior to larval-pupal transformation in serval other insects (Turnbull and Howells, 1980). In both the treatments, the last stage of analysis had pronounced staining of protein bands. However, the ecdysteroid deficiency in these larvae led to the failure of synthesis of proteins typical of the pupal cuticle. At 120 h, four additional bands were recorded in plumbagin-treated insects, whereas only a total of five bands were well pronounced due to azadirachtin treatment compared to that of control. This suggests variation in the action of two test compounds on the cuticular profile which has to be elaborately studied.

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Descriptions of a New Genus *Nigropria* and a New Species of *Aneuropria* Kieffer (Diapriidae : Proctotrupoidea : Hymenoptera) from India

K. Rajmohana*[1] and T. C. Narendran[2]

[1] *Zoological Survey of India, Calicut, Kerala, India 673002*
[2] *Systematic Entomology Laboratory, Department of Zoology, University of Calicut, Kerala, India 673635*

ABSTRACT: A new genus of Diapriinae, *viz.*, *Nigropria* with type species, *Nigropria compressa* sp. nov., and a new species namely *Aneuropria kairali* (Diapriidae, Proctotrupoidea) are described and illustrated.

KEYWORDS: New genus, New species, Diapriidae, India.

INTRODUCTION

The following genera of Diapriinae (Diapriidae, Proctotrupoidea) known from the Oriental Region were keyed by Rajmohana and Narendran (1998): *Aneurhynchus* Westwood, *Aneuropria* Kieffer, *Alareka* Rajmohana & Narendran, *Basalys* Westwood, *Calicuta* Rajmohana & Narendran, *Claudivania* Huggert, *Clinopria* Kieffer, *Coptera* Say, *Cyathopria* Kieffer, *Diapria* Latreille, *Dilobopria* Kieffer, *Dolichopria* Kieffer, *Entomacis* Foerster, *Hemigalesus* Kieffer, *Hoplopria* Kieffer, *Monelata* Foerster, *Neurogalesus* Kieffer, *Odontopria* Kieffer, *Oxypria* Kieffer, *Paramesius* Westwood, *Pleuropria* Kieffer, *Psilus* Panzer, *Scapopria* Kieffer, *Spilomicrus* Westwood, *Stylopia* Kieffer, *Trichopria* Ashmead, *Vadana* Rajmohana & Narendran and *Xyalopria* Kieffer.

Among these all genera except *Aneurhynchus*, *Clinopria*, *Scapopria*, *Pleuropria*, *Stylopia*, *Neurogalesus*, *Hemigalesus*, *Hoplopria*, *Diapria* and *Dilobopria* have been reported from the Indian subcontinent. Since genus *Nigropria* is rather unique in many characters and cannot be included under any of the above mentioned genera, it is described hereunder as new to science. Genus *Aneuropria* Kieffer had been represented hitherto in the Indian Region as well as the Oriental by just a single species, *viz.*, *Aneuropria nilgiriensis* (Sharma, 1979). So this new species namely *A. kairali* forms the second record. Since description of *A. nilgiriensis* was based on male, *A. kairali* sp. nov. forms the first record of female of the genus from India as well as Oriental.

*Corresponding author

Genus Nigropria gen.nov.

Type species: *Nigropria compressa* sp. nov., by monotypy.

Diagnosis

Head and body shining black. Orbital carina distinct with a wavy inner edge and fully encircling lower margin of eyes. Eyes situated high up in face. Antenna thirteen segmented in female. Mouth parts hypognathous. Mesosoma in lateral view not convex, but steep. Pronotum with pointed angular anterolateral corners. Notauli deep, complete and widely diverging in front. Scutellum trough shaped, with two small anterior foveae. Propodeum with a deeply excavated posterior margin and longitudinal rugulae, radiating from basal emargination. *sm* distinct, *m* as well as *st* seen as a speck; faint traces of radial present; *basalis* sharp, extending up to *cu*; a longitudinal whitish non-hairy streak towards a little below to median and extending towards wing margin. *swm* not reaching frenum of hind wing. Metasoma smooth, shiny and dorso-ventrally flattened; T2 with its basal margin, distinctly concave.

Morphological characters distinguishing
Nigropria gen. nov., *Spilomicrus* Westwood and *Odontopria* Kieffer

No	Characters	*Nigropria* gen. nov.	*Spilomicrus*	*Odontopria*
1	Punctae on dorsal head	Absent	Absent	Deep distinct and rounded
2	Orbital carina	Distinct	Absent	Distinct
3	Longitudinal carina on dorsal head	Absent	Absent	Often a median carina extending from hind occiput
4	Mesosoma in lateral view	Steep and not convex	Distinctly convex	Distinctly convex
5	Anterior pronotum	Acutely angular lateral corners	Feebly angular lateral corners	lateral corners angular
6	Propleuron	With a deep concavity	Without a deep concavity	With a deep concavity
7	Metasoma	Distinctly dorso-ventrally flattened	Not flattened dorso-ventrally	Not flattened dorso-ventrally

Remarks

The steep or erect mesosoma and a dorso-ventrally flattened metasoma are rather peculiar for this genus (metsaoma more than 2.6x as wide as high. Such a combination of characters, has never been encountered in Diapriids.

The genus resembles *Spilomicrus* Westwood and *Odontopria* Kieffer in possessing a thirteen segmented antenna, without an abrupt clava. All the three have very similar wing venation too.

Etymology

The genus name '*Nigropria*', means 'Black wasp' (feminine gender) and species name '*compressa*' refers to the dorso-ventrally compressed metasoma.

***Nigropria compressa* sp. nov. (Figs 1–5)**

Female

Length 1.94 mm. Head and body shining black. antennae brownish black. Eyes silvery black. Coxa, trochanter and femur brown with a black tinge, tibia and tarsi brown and tarsal claws deep brown. Tip of metasoma including ovipositor brown. Wings not infuscate, veins deep brown. Body pubescence dull white; marginal fringe of wings brown.

Head

When viewed dorsally roughly globose, with a gently arched vertex. HH : HL = 26 : 24.6. orbital carina distinct with a wavy inner edge and fully encircling lower margin of eyes, passing through median of temples. Ocelli large; OOL : OD : POL = 2 : 1.5 : 3; Pubescence scattered and erect. Occipital flange distinct, not step-like and dorsally with a few median punctae. Frons feebly convex and straight when viewed laterally. Eyes bare, situated high; max. eye width: malar space = 14.5. Malar sulcus present with carinate inner margin; genal carina distinct; postgenal cushion of hairs scanty; temples receding beneath eyes and with a gentle curve towards occiput. Mouthparts hypognathous, mandibles bidentate, clypeus and toruli distinct. Antenna in female with thirteen segments and without an abrupt clava, but gradually thickened; 1.1.5.6. F3 to F5 globose; antenna densely hairy; antennal setae longer than distal width of *sc*; *sc* 3.27x as long as wide; terminal segment 1.8x as long as penultimate; proportion of length and width of antennal segments from *sc* to F11 being, 18 : 5.5, 6.8 : 4.5, 8 : 4.1, 5.7 : 4.1, 4.8 : 4.3, 4.8 : 4.5, 4.8 : 5, 4.8 : 5, 4.8 : 6, 4.8 : 6.8, 4.8 : 6.8, 4.8 : 6.8, 8.8 : 6.

Mesosoma

Unusually long, L : B = 44 : 30.5, 1.44x as long as wide and a little wider than head; in lateral view distinctly non-convex, but steep. Cervix bare with a few deep punctae. Pronotum with pointed angular anterolateral corners, pronotal cushion of hairs sparse. Mesonotum gently arched anteriorly; notauli depp, complete and widely diverging in front, in base separated by a distance of 4x its diameter; humeral sulcus absent. TSS almost straight. Scutellum widely trough shaped, with two small adjacent and slanting anterior foveae; scutellar sheath broad. Dorsellum simple, wide, with three small equidistant buds, replacing carinae. Propodeum with a deeply excavated posterior margin and with many longitudinal rugulae radiating from basal emargination; almost bare except sparse hairs laterally; median carina on propodeum not produced into spine or tooth. Propleuron with inwardly depressed sides, forming a deep depression, bordered by a thick stout carina in lower margin. Mesopleuron smooth, sternaulus

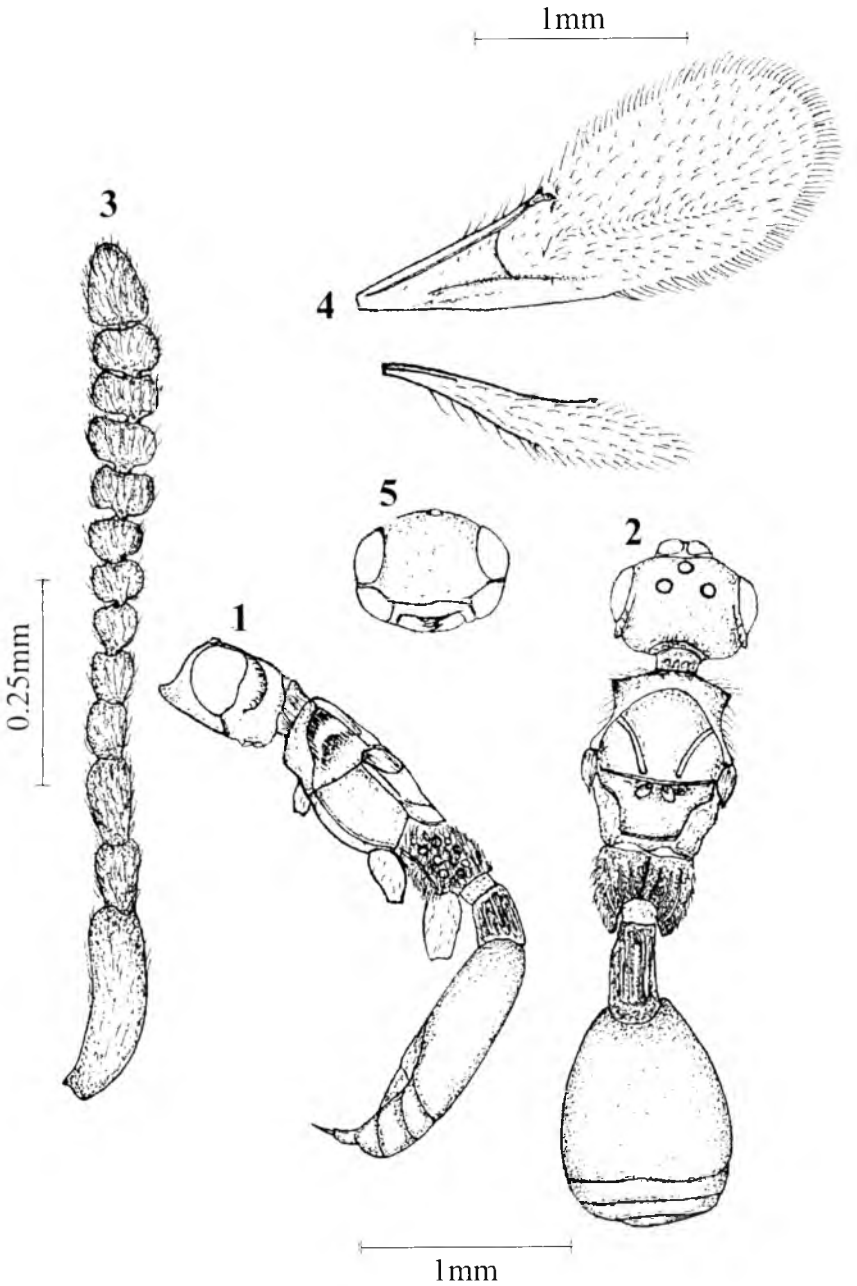


Plate 1. ***Nigropria compressa*** sp. nov. female: 1. Body profile, 2. Body (D.V), 3. Antenna, 4. Forewing and Hindwing, 5. Head (A.V)

distinct. A longitudinal furrow extending beneath tegula throughout mesopleuron. Metapleuron with coarse reticulation and a pair of small longitudinal carinae near the base of hind coxa; a sparse covering of fine decumbent hairs, partly hiding underneath sculpture. Legs simple, tarsal claws bifid. fore wing L : B = 9.6 : 3.45; veins reaching 0.46 of wing length; *sm* distinct, *m* and *st* as a speck; faint traces of radial present; *basalis* sharp extending up to *cu*; a longitudinal whitish non-hairy streak a little below to median, extending towards wing margin.

Mesosoma

Petiole robust, with irregular widely placed longitudinal striations; not hairy dorsally, but laterally with sparse fine short hairs; 1.67x as long as wide, excluding nucha. Metasoma smooth, shiny and dorsoventrally flattened, 2.6x as wide as high, MTW : MTH = 34 : 13; T2 with its basal margin distinctly concave, but not medially incised. T2 extending to 0.78 of dorsal metasoma (excluding its curved tip), distally incurved, ovipositor exserted; end tergites densely hairy.

Male Unknown
Host Unknown

Holotype: Female

India, Kerala; Pamba; Coll. P. M. Sureshan (ZSI, Calicut) 22-ii-1997. The type for the time being is retained at ZSI, Calicut.

Genus *Aneuropria* Kieffer 1905

Type *Aneuropria clavata* Kieffer (*Polypeza foersteri* Kieffer), by monotypy.

Only two species *viz.*, *A. clavata* Kieffer and *A. nilgiriensis* Sharma have been hitherto reported under Anureopria.

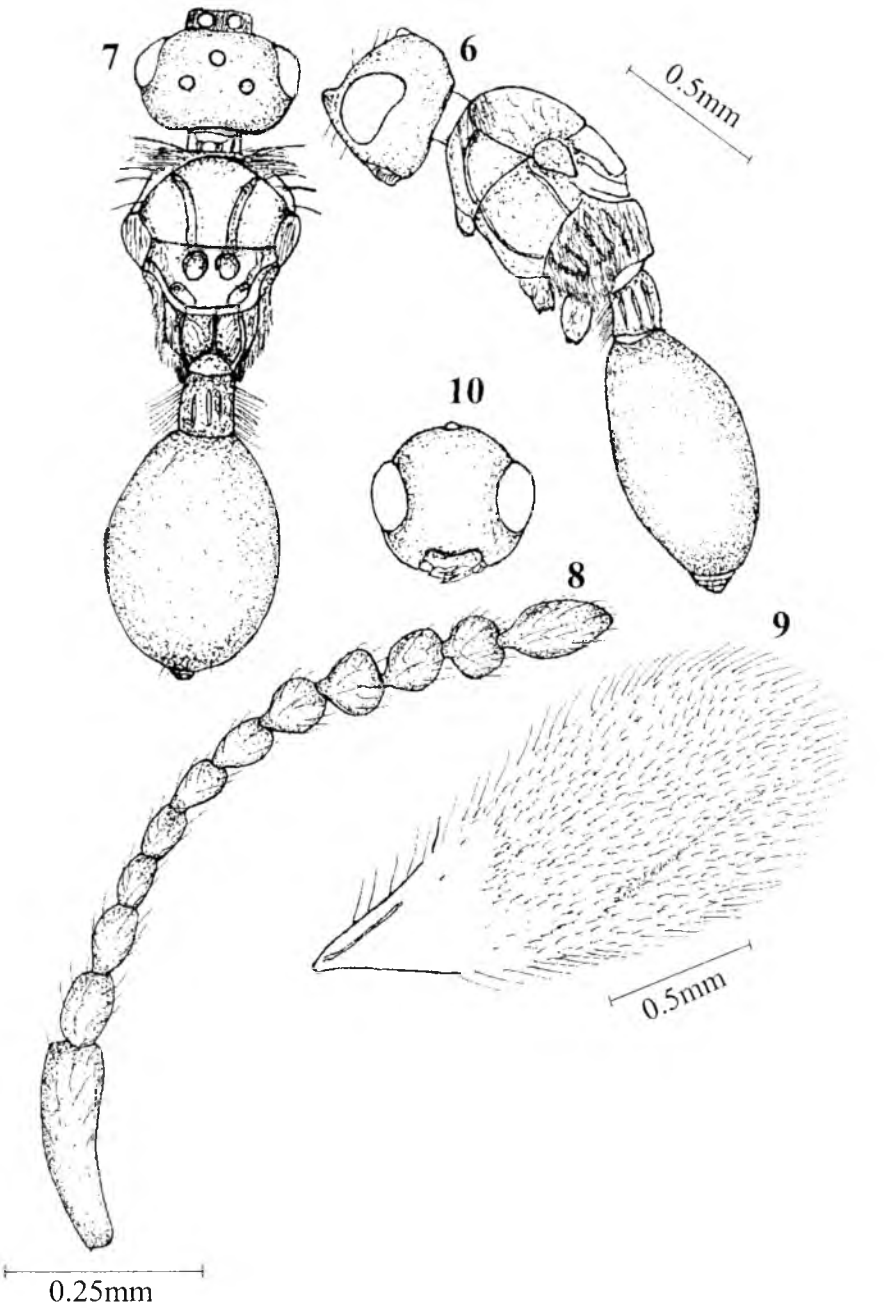
Diagnosis

Female antenna twelve- segmented and male antenna fourteen-segmented. Mouthparts hypognathous. Post genal cushion of hairs and pronotal collar of hairs dense. Notauli complete. Scutellum with two adjacent anterior foveae and two longitudinal foveae on scutellar sheath. Fore wings without veins, only *sm* represented basally. T2 with or without a basal median longitudinal furrow, occupying almost whole of dorsal metasoma.

***Aneuropria kairali* sp. nov. (Figs 6–10)**

Female

Length 1.48 mm. Head and body shining black. Eyes bronze, basal antennal segments including scape reddish brown. Terminal 6 segments brownish black. Legs brown. Wings transparent. Antennal pubescence and marginal fringe of wings brown. Body pubescence dull white.



Aneuropria kairali sp. nov. female: 6. Body profile, 7. Body (D.V), 8. Antenna, 9. Forewing, 10. Head (A.V)

Head

Smooth and shiny; when viewed from above distinctly wider than long, with scattered sparse hairs. antennal shelf well above vertex. Eyes pear-shaped, not bulging laterally, with sparse erect hairs; eyes without any orbital carina. Temples beneath eyes sub-parallel, gradually sloping and curving towards occiput. Ocelli arranged in a wide triangle, OOL : OD : POL = 1 : 0.5.2; occipital flange distinct with very minute adjacent punctae. Frons almost bare, post genal cushion dense, genal carina absent; mandibles bidentate, malar sulcus lacking; max. eye width: malar space = 4.1; clypeus and tentorial pit distinct. Antennal insertion high, higher than level of upper orbital margin; antenna twelve-segmented; A.F = 1.1.4.6; terminal six segments gradually enlarged, without an abrupt clava; A12 without a ventral pit. F6 to F9 bead like; terminal siz segments more hairy; *sc* 3.31x as long as wide, F10 1.7x as long as F9; proportions of antennal segments form *sc* to F10 being 20.5 : 6.2, 8 : 4.9, 7 : 4, 5.2 : 3.5, 5.5 : 4, 5.2 : 4, 6 : 4.5, 6.5 : 5.2, 6.5 : 6.4, 6.5 : 6, 11 : 6.

Mesosoma

L : B = 4.1 : 3.65, a little wider than head; dorsal cervix with deep wide striae. Pronotal cushion of hairs moderate, long, concealing upper border of pronotum; anterior pronotum weakly angular. Notauli complete and diverging in front, separated at base by 3x its diameter. Humeral sulcus absent. TSS distinct. Scutellum rather wide, with two adjacent slanting oval foveae; scutellar sheath bordered laterally by small longitudinal foveae. Metanotum with three faint longitudinal carinae, a median and a pair of laterals. Propodeum with a distinct median carina, not raised to a spine, flanked on either side by two pairs of lateral longitudinal carinae, outer pair rather bent and inner pair erect; deeply emarginate basally, posterior emargination of propodeum laterally with tooth-like process extending downward; median propodeum with fine hairs arranged transversely. Propleuron and mesopleuron bare, a bent carina encircling upper propleuron. Sternaulus distinct. Metapleuron with fine short adpressed hairs, concealing irregular longitudinal striations and reticulations underneath. Legs normal, fore tibia without a false spur, densely hairy after proximal one-fourth. Fore wings, large L:B = 11.24.7; *sm* incomplete and reduced to stump reaching only 0.22 of wing length, other veins absent; marginal fringe of fore wings well developed.

Metasoma

Petiole 1.5x as long as wide; hairy laterally, with distinct deep longitudinal straie. Smooth and shiny, 1.3x as wide as high; MTW: MTH = 3.65:2.8; T2 extending to almost tip, 0.96 of metasoma; base only a little concave and without a median incision; sparsely hairy towards tip.

Male Unknown
Host Unknown

Holotype: Female

India, Kerala, Malampuzha; Coll. P. M. Sureshan (ZSI, Calicut); 18-3-1999. The type for the time being is retained at ZSI, Calicut.

Remarks

This forms the first report of female *Aneuropria* from the Oriental as well as the Indian Region. Earlier Sharma (1979) had described a male of *A. nilgiriensis* from India. *A. clavata* Kieffer and *A. nilgiriensis* Sharma possess a median incision on T2. But *A. kairali* sp. nov. lacks a basal median longitudinal incision on T2. In all other characters, this new species agrees with Kieffer's generic description of *Aneuropria*, erected based on monotypy. thus in *Aneuropria*, T2 may be with or without a basal median longitudinal furrow.

Abbreviations

A.F = antennal formula; F1–F10 = flagellomeres 1–10; L : B = ratio of length and breadth; OD = ocellar diameter; OOL = ocellocular length; POL = post ocellar length; sc = antennal scape; TSS = Trans scutellar suture; *m* = marginal vein; *sm* = submarginal vein; *st* = stigmal vein; cu = cubitus vein; T2 = 2nd metasomal tergite; HH = head height (Measured between top of ocelli and closed edge of bottom mandibles in lateral view); HL = head length = (Measured between anterior edge of antennal shelf and posterior edge of occiptial flange) MTH = metasomal height (measured between top of T2 and bottom of S2 in lateral view); MTW = metasomal width (measured across T2 dorsally). ZSI = Zoological Survey of India.

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Observation of Low Tolerance to *Bacillus thuringiensis* var *israelensis* in *Culex quinquefasciatus* Resistant to *Bacillus sphaericus*

S. Poopathi*, Lalitha Kabilan, T. R. Mani, D. Raghunatha Rao and G. Baskaran

Centre for Research in Medical Entomology (ICMR), 4, Sarojini street, Chinna Chokkikulam, Madurai 625002, India
Email: icmrcrme @ren.nic.in

ABSTRACT: A variety of formulations of *Bacillus sphaericus* (*Bs*) and *Bacillus thuringiensis* var *israelensis* (*Bti*) have been studied for mosquito control under laboratory and field conditions. However, there are reports of *Bs* - resistant *Culex quinquefasciatus* of Californian strain developing tolerance to *Bti*. we have subjected laboratory reared *Bs*-resistant *Cx. quinquefasciatus* to selection pressure with *Bs* 1593M strain for five years continuously and tested whether they are cross-resistant to *Bti* (IPS-82). We have observed a low level of tolerance to *Bti* among the *Bs* selected resistant (GR) when compared to *Bs* unselected (GS) and normal (MS) strains of *Cx. quinquefasciatus*. Tolerance was continued to be seen in four consecutive filial generations (65th to 68th) of larvae tested and there was no generation dependent variation in the tolerance level. The tolerance level did not differ significantly in all lethal concentrations studied. Therefore, to manage microbial resistance in mosquitoes, alternate measures are indicted.

KEYWORDS: *Bacillus sphaericus*, *Bacillus thuringiensis* var *israelensis Culex quinquefasciatus*, resistance, resistance management.

INTRODUCTION

Bacillus sphaericus Neide (*Bs*) *Bacillus thuringiensis* var *israelensis* de Barjac (*Bti*) are most important spore forming soil bacteria widely applied for mosquito control operations in the field (Mulligan *et al.*, 1978, 1980; Pantuwatana and Youngvanitsed, 1984; Kramer, 1984; Mulla, 1985, 1986; Mulla *et al.*, 1988; Goettel *et al.*, 1992; Yadav *et al.*, 1997). The binary toxin (42 and 51 Kilodalton proteins) from *Bs* and the multiple toxin (27, 65, 128 and 135 kDa proteins) from *Bti* are the most important toxins that interact and produces a complex effect on the mosquito larvae (Wu and Chang, 1985; Federici *et al.*, 1990; Broadwell *et al.*, 1990; Poncet *et al.*, 1995). The *Culex* spp. are more susceptible to binary toxin than *Anopheles* spp. and *Aedes* spp. (de Barjac, 1990).

*Corresponding author

However, recent reports point out development of high level resistance by *Culex* spp. to binary toxin of *Bs* (Georghiou *et al.*, 1992; Rodcharoen and Mulla, 1994; Rao *et al.*, 1995; Silva and Regis, 1997) and a low level of resistance to multiple toxins of *Bti* (Goldman Ira *et al.*, 1986; Wirth and Georghiou, 1997). Cross-resistance to *Bs* strains in *Culex quinquefasciatus* was recently reported (Poopathi, 1995). A Californian strain of *Cx. quinquefasciatus* also exhibited cross-resistance to *Bs* strains and low tolerance to *Bti* (Rodcharoen and Mulla, 1996). To understand microbial resistance in mosquitoes, it is essential to differentiate the terms of resistance, cross-resistance and tolerance. The resistance is one when the larvae are refractory against a biopesticide. The cross-resistance is the one when a resistant strain shows cross resistance to other strains of the biopesticide also. Similarly, the tolerance is the one that is neither cross-resistant nor susceptible but marginally tolerant to the biopesticide (Rodcharoen and Mulla, 1994, 1996; Rao *et al.*, 1995). In the present study, we have evaluated the tolerance levels of *Bacillus sphaericus* (1593M) resistant *Cx. quinquefasciatus* larvae to *Bacillus thuringiensis* var *israelensis* (IPS-82).

MATERIALS AND METHODS

Background

A large scale *Culex quinquefasciatus* control field trial was carried out in an area of 8 km^2 in Gandhinagar (Kochi, Kerala, S. India) with a formulation of *Bacillus sphaericus* (1593M) (produced by Centre for Biotechnology, Anna University, Chennai and named as Biocide-S) over a period of two years. A good control of breeding (>90% mortality) was achieved during the first year of control operation and in the next year, satisfactory control was not obtained despite good coverage of biolarvicide spraying (Mani, 1992). It was, therefore, suspected that the poor results could be due to the development of resistance in the field. Samples of larvae collected from biocide treated area were transported to the laboratory, bioassayed with *Bacillus sphaericus* (SPH-88) and confirmed a high level of resistance (Rao *et al.*, 1995). The resistant strain in the laboratory was maintained by subjecting to moderate selection pressure with *Bacillus sphaericus* (1593M) at each generation (early third instar larvae were treated at a concentration to yield 50% mortality (LC_{50}) in 48 hrs and the surviving larvae were reared to next generation). At F14, the resistant strain was cultured in two lines and in one line, the application of selection pressure was continued and maintained as field collected selected line (selected resistant strain, GR) and in another line, selection pressure was discontinued and maintained as field collected unselected line (unselected resistant strain, GS). GR strain was subjected to selection pressure continuously for the last five years in the laboratory. Besides these larvae, larvae collected from Madurai Urban area, where no biocide was sprayed earlier, was also reared in the laboratory and used as Madurai susceptible strain (MS) in the present study.

TABLE 1. Tolerance levels of *Bacillus sphaericus* (1593M) resistant *Culex quinquefasciatus* larvae to *Bacillus thuringiensis* var *israelensis* H14 (IPS-82)[a]

Generation	Strains[b]	Intercept	Slope ± SE	LC_{50}(mg/L)	LC_{90}(mg/L)	LC_{95}(mg/L)	X^2 (df)	TR (at LC_{50})[f]	TR (at LC_{90})[f]	TR (at LC_{95})[f]
	GR	9.78	2.43 ± 0.6	0.011 (0.014 – 0.0084)[c]	0.036 (0.054 – 0.025)[d]	0.051 (0.087 – 0.03)[e]	12.03(5)			
	GS	10.12	2.06 ± 0.6	0.003 (0.05 – 0.002)	0.0139 (0.028 – 0.007)	0.021 (0.052 – 0.008)	18.05(4)	3.7 (4.2 – 2.6)	2.6 (3.6 – 1.9)	2.4 (3.6 – 1.7)
65	MS	9.77	1.87 ± 0.6	0.0028 (0.005 – 0.0016)	0.014 (0.028 – 0.0067)	0.021 (0.056 – 0.008)	16.08(4)	3.9 (5.3 – 2.8)	2.6 (3.7 – 1.9)	2.4 (3.7 – 1.6)
	GR	9.75	2.52 ± 0.5	0.013 (0.014 – 0.011)	0.041 (0.051 – 0.033)	0.05 (0.076 – 0.043)	6.58(4)			
66	GS	10.12	2.06 ± 0.6	0.0033 (0.0054 – 0.002)	0.014 (0.028 – 0.0069)	0.02 (0.052 – 0.008)	18.05(4)	3.9 (5.5 – 2.6)	2.9 (4.8 – 1.8)	2.7 (5.4 – 1.5)
	MS	9.19	1.66 ± 0.5	0.0029 (0.0059 – 0.0015)	0.0175 (0.048 – 0.0063)	0.029 (0.11 – 0.007)	19.84(4)	4.5 (7.3 – 2.4)	2.3 (5.2 – 1.1)	2.0 (4.1 – 0.3)
	GR	9.9	2.59 ± 0.9	0.013 (0.018 – 0.009)	0.0402 (0.073 – 0.022)	0.056 (0.119 – 0.026)	8.66(3)			
67	GS	9.21	1.66 ± 0.6	0.0029 (0.06 – 0.0013)	0.017 (0.056 – 0.0053)	0.028 (0.139 – 0.006)	25.57(4)	4.5 (6.9 – 3.0)	2.4 (4.2 – 1.3)	2.0 (4.5 – 0.9)
	MS	9.46	1.76 ± 0.6	0.0029 (0.0063 – 0.0014)	0.016 (0.048 – 0.005)	0.025 (0.111 – 0.006)	26.2(4)	4.5 (6.4 – 2.9)	2.5 (4.5 – 1.5)	2.2 (4.3 – 1.1)
	GR	9.61	2.42 ± 0.6	0.013 (0.016 – 0.009)	0.0425 (0.058 – 0.027)	0.059 (0.109 – 0.033)	13.98(5)			
68	GS	9.21	1.66 ± 0.6	0.0029 (0.0062 – 0.0013)	0.0171 (0.052 – 0.0056)	0.028 (0.127 – 0.006)	23.22(4)	4.5 (7.6 – 2.6)	2.5 (4.8 – 1.3)	2.1 (5.3 – 0.9)
	MS	8.56	1.33 ± 0.3	0.0021 (0.0034 – 0.0013)	0.0191 (0.047 – 0.0078)	0.036 (0.113 – 0.011)	26.62(6)	6.2 (7.6 – 4.7)	2.2 (3.5 – 1.4)	1.6 (3.0 – 1.0)

a Bacillus thuringiensis var *israelensis* H14 (IPS-82): Titre = 1700 ITU/mg toxin. [b] GR = *Bs* selected resistant strain; GS = *Bs* selected resistant strain; GS = *Bs* unselected restistant strain; MS = normal susceptible strain. [c,d,e] 95% Fiducial limits of upper and lower at LC_{50}, LC_{90} and LC_{95} levels. [f] Tolerance ratio (TR) = Experimental vales (GR) ÷ Control values (GS/MS)

Mosquito colony

Cx. quinquefasciatus larval strains (GR, GS and MS) were reared in the laboratory at ambient laboratory temperature (29–31 °C) in enamel trays providing yeast and dog biscuit at the ratio of 40 : 60 in water as nutrient source. Pupae were allowed to emerge in cages and the adults were sexed. Females were provided with blood meal from live chicken and males with 5–10% glucose solution through cotton pads and water soaked raisin. Adults were allowed to oviposit in water in enamel cups kept inside emergence cages. Freshly hatched larvae from egg rafts of the larval strains were cultured individually as cyclic colonies on each filial generation.

Bioassays

In the present study, newly emerged fourth instar larvae from 65th to 68th filial generations were used for bioassays. Lyophilized bacterial culture of *Bacillus thuringiensis* var *israelensis* H14 (IPS-82) (titre : 1700 International toxic units/mg toxin) provided by Dr. J.-F. Charles, Institute Pasteur, Paris, France was used. Titration and preparation of stock solution from *Bacillus thuringiensis* var *israelensis* and bioassays were carried out according to World Health Organization protocol (1985). In the present study, 50 mg of *Bti* spore/crystal toxins was homogenized in deionized water and stock solution was prepared as 50 mg *Bti*/L. Subsequent dilutions were approximately made. The aliquot of appropriate dilutions ranging from 0.035 to 0.003 mg *Bti*/L and from 0.05 to 0.0008 mg/L (9 doses) were used for resistant (GR) and susceptible (GS, MS) strains respectively. Bioassays were conducted in disposable paper cups (200 ml capacity). To 150 ml of test medium prepared by adding appropriate volume of *Bti* toxin, twenty five freshly moulted fourth instar larvae of GR, GS and MS strains were introduced individually in each test concentration. Larval food was not given for the test larvae as recommended by World Health Organization (1985). At each tested concentration five trials were made and each trial consisted of three replicates. Larvae exposed to water served as control. Larval mortality was observed after 24 hrs of exposure. Moribund larvae if any, were counted as dead. The control larval mortality was scored after 24 hr and corrected for any control mortality by adapting Abottt's (1925) formula:

$$\text{Corrected Control Mortality} = \frac{\%\text{ test mortality} - \%\text{ control mortality}}{100 - \%\text{ control mortality}} \times 100$$

The software package ASSAY (provided by Dr. C. F. Curtis, London School of Tropical Medicine and Hygiene, U.K) was used for dosage mortality regression analysis. Tolerance ratio (TR) at LC_{50}, LC_{90} and LC_{95} levels were calculated by the method of Robertson and Preisler (1992).

$$\text{Tolerance Ratio (TR)} = \frac{LC_{50}/LC_{90}/LC_{95} \text{ of } Bs \text{ - resistant strain}}{LC_{50}/LC_{90}/LC_{95} \text{ of } Bs \text{ - susceptible strain}}$$

RESULTS AND DISCUSSION

In the present study, we have evaluated the tolerance level to *Bacillus thuringiensis* var *israelensis* H14 (IPS-82) by *Cx. quinquefasciatus* larvae which were resistant to *Bacillus sphaericus* (1593M) obtained by selection pressure for the last five years in the laboratory mosquito colony. Table 1 provides data on tolerance ratio (TR) between *Bs* selected resistant (GR) and unselected resistant (GS) and normal (MS) strains i.e. GR vs GS and GR vs MS in four consecutive filial generations (from 65th to 68th). As shown in the table, at LC_{50} level, the tolerance ratio between GR and GS were 3.7, 3.9, 4.5 and 4.5 folds for 65th, 66th, 67th and 68th filial generation respectively. The tolerance ratio between GR and MS were 3.9, 4.5, 4.5 and 6.2 folds for these four filial generations respectively. At LC_{90} level, the tolerance ratio between GR and GS; GR and MS were 2.6, 2.9, 2.4, 2.5; 2.6, 2.3, 2.5, 2.2 folds respectively. At LC_{95} level also, the tolerance ratio between GR and GS; GR and MS were almost the same and varied between 1.6 to 2.7 folds for the test generations. The variation in the tolerance ratio was not significantly seen among different filial generations at LC_{50}, LC_{90} and LC_{95} levels. It is expected that there may not be any variation in tolerance ratio, since the resistant level to *Bacillus sphaericus* by these four generations were similar (data not shown). Statistically no significant difference in tolerance ratio was observed in all lethal concentration (LC_{50}, LC_{90} and LC_{95}) levels since the fiducial limits were overlapping. Thus our results suggest that tolerance at a low level to *Bti* is possible in *Bs* resistant *Cx. quinquefasciatus* larvae collected from Gandhinagar (Kochi, Kerala) area where Biocide-'S' was treated for two consecutive years.

Studies elsewhere have reported (Rodcharoen and Mulla, 1996) low level of tolerance to *Bti* by Californian strain of *Cx. quinquefasciatus* (2 to 3 folds at LC_{50} and LC_{90} levels) resistant to *Bacillus sphaericus* (2362) strain. Thus our results are in accordance with the findings of their study. These results signal for caution and search for alternate measures to manage microbial resistance in mosquitoes.

A number of laboratories have been actively involved in the exploration of mode of action of bacterial toxin in the gut of mosquito larvae of resistant and susceptible strain to mange resistance (Davidson, 1988, 1989; Hofmann *et al.*, 1988; Nielsen-LeRoux and Charles, 1992; Nielsen-LeRoux *et al.*, 1995, 1997; Charles *et al.*, 1997). The authors reported through *in vitro* binding assays that binding of active toxin from *Bacillus sphaericus* was possible with specific receptors present in the midgut brush border membrane of *Bs* susceptible larvae. Whereas, loss or reduction of functional receptors in binding site of midgut brush border membrane led to resistance in mosquitoes. So we assume that tolerance to *Bti* in *Bs* resistant *Cx. quinquefasciatus* was attributed by the toxin that share further loss or reduction of binding sites in the midgut. However, the exact mechanism of resistance still needs to be explored to develop alternate measures to manage microbial resistance in mosquitoes. Combination of *Bacillus sphaericus* and neem based bio-pesticide have been successfully used to overcome the resistance in *Bs* resistant larvae (Poopathi *et al.*, 1997). Synergistic larvicidal activities of biopesticides of natural products against

different mosquito species has to be evaluated to overcome the problem of microbial resistance in mosquito control operation.

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A Study of Insects of Terrestrial Origin Over North Arabian Sea

S. C. Pathak*, Vandana Kulshrestha and Arun Kumar Choubey†

Department of Post Graduate Studies and Research in Biological Sciences, Rani Durgavati University, Jabalpur, (MP) 482001, India
Email: pathaksc@hotmail.com

ABSTRACT: Analysis of airborne–insects at a height of 18 to 22 meters above sea level, over north Arabian sea in March–April 1996, revealed a variable aerial density of insects over various sampling stations, which could be clubbed into five clusters. The density depended upon the distance of the nearest landmass(es) *in wind direction*. The density was higher in cluster closest to the land; also it was higher in clusters which received winds from the Indian shores as compared to the cluster that received winds from the Persian Gulf area. One cluster that received winds from both the sources, showed not only higher density but also higher species diversity and richness with all the species contributing equally to the aerial density. The bulk of the trapped insects belonged to Hymenoptera, Hemiptera and Diptera. Coleopterans were also present in some catches. Trichoptera, Neuroptera, Lepidoptera and Orthoptera only marked their presence.

KEYWORDS: Insects, Arabian sea, Insect population, Species diversity.

INTRODUCTION

Insects are known to cross vast oceans and reach new continents, covering distances as large as 3000 km (Bowden and Johnson, 1976). These insects, mostly small, size varying from 0.4 to 15 mm, get caught in thermal currents when the land gets heated and reach variable heights upto 5000 m and are then carried by winds in whichever direction they flow, including over the oceans. This migration is mostly passive. There is no feeding during this journey and the insects survive on whatever energy reserves were present at the time of getting caught in the thermal currents (Cheng, 1976). Most of these airborne insects over the oceans, ultimately fall in sea water where they are either predated upon by plankton feeders or get killed and sink to the bottom within 48 hours, contributing to oceanic biomass.

Airborne insects of terrestrial origin have mostly been studied in Pacific (Gressitt and Nakata, 1958; Gressitt *et al.*, 1960; Harrell and Yoshimoto, 1964; Harrell and

*Corresponding author
†Deceased

Holzapfel, 1966) and Atlantic (Clagg, 1966) oceans, besides the North sea and English channel (Hardy and Cheng, 1986). Not much is known about insects of terrestrial origin over any part of the Indian ocean except for a report of the Danish expedition 'Galathea' of 1950–52 which trapped some insects along the eastern coast line of India in the Bay of Bengal (Yoshimoto *et al.*, 1962) and some reports of some of the present authors (Pathak and Parulekar, 1988; Pathak, 1998; Pathak *et al.*, 1999). Presented here are some observations on the population of insects of terrestrial origin over the northern part of the Arabian sea.

MATERIAL AND METHODS

Airborne insects were collected between Latitude 16 °N and 20 °N and Longitude 68 °E to 72 °E, aboard ORV *Sagar Kanya*, in the course of cruise #111 in March–April 1996, using specially designed multiple–net insect trapping systems for use on ships (patent pending). Three conical nets were hoisted one above the other in each system covering a total height of 4 meters, between 18 m and 22 m above the sea surface. The nets always faced winds and were emptied twice every day at 0730 and 1800 h, the catches representing night and day trappings respectively. The locations of the ship where the nets were emptied are referred as the sampling stations (although the ship continued to move during the process of emptying). The coordinates of the sampling stations were recorded.

Insects recovered were immediately sorted and kept in glass vials in 80% alcohol. Identification, mainly to families, was carried out later in the laboratory.

The data of the insect population were analysed and indices worked out for species diversity, richness and evenness (Shannon and Weaver, 1963).

The aerial density of insects in the study area was calculated by dividing the number of insects trapped by the product of distance travelled, width of the sampling i.e. 1.5 m (the diameter of the mouth of each net being 75 cm; two nets-one on each side of the bridge of the ship taken into account) and the height i.e. 4 m. The wind speed was assumed to be zero for this calculation.

The wind direction and speed were determined with the help of wind vane and portable anemometers (OGAWA SEIKI, Japan) and noted every day at 0700, 1400 and 1800 h and corrected for the ship speed and direction of movement using standard mariner's tables. This data and the data of the wind speed and direction on the west coast of India provided by the National Data centre for Indian Ocean, National Institute of Oceanography, Dona Paula (Goa) were used for computing the generalised pattern of wind flow in the area.

RESULTS AND DISCUSSION

A total of 2301 insects belonging to 8 orders, 47 families and 173 different taxa were trapped during the cruise (Table 1). Although identification to species level was not carried out, it was confirmed that there were 173 'recognisable taxonomic units'. Hymenoptera constituted the largest group (47%), followed by Hemiptera (25% +)

TABLE 1. Airborne insects trapped over north Arabian sea in March–April 1996. Cruise #111, ORV *Sagar Kanya*

Sl. No.	Order	Family	Number of Species	Number of Individuals	% of the total catch
1.	Hymenoptera				46.9
		Braconidae	4	18	
		Eulophidae	14	36	
		Pteromalidae	09	83	
		Encyrtidae	06	58	
		Agaontidae	17	854	
		Mymaridae	03	07	
		Formicidae	04	12	
		Elasmidae	01	01	
		Vipionidae	01	05	
		Halichidae	01	04	
		Scelionidae	01	01	
		Chalcididae	01	01	
2.	Hemiptera s.o Homoptera				25.3
		Chermidae	04	25	
		Fulgoridae	07	208	
		Jassidae	05	58	
		Cercopidae	01	04	
	s.o. Heteroptera				
		Coreidae	15	278	
		Miridae	01	01	
		Lygaeidae	01	04	
		Berytidae	02	04	
		Ploiariidae	01	02	
3.	Trichoptera				0.52
		Leptoceridae	04	11	
		Hydroptilidae	01	01	
4.	Neuroptera				0.43
		Chrysopidae	01	10	
5.	Lepidoptera				0.13
	Microlepidoptera *unidentified*		01	03	
6.	Orthoptera	Gryllidae	01	01	
7.	Diptera				23.9
	s.o.Nematocera				
		Chironomidae	06	175	
		Cecidomyiidae	09	86	
		Ceratopogonidae	01	01	
	s.o. Brachycera				
		Dolichopodidae	01	01	
		Lonchopteridae	01	04	

Table I – *continued*

S. No.	Order	Family	Number of		% of the total catch
			Species	Individuals	
	s.o. Cyclorrhapha				
		Chloropidae	12	93	
		Drosophilidae	03	49	
		Agromyzidae	07	119	
		Phoridae	02	08	
		Tachinidae	02	02	
		Muscidae	02	08	
		Anthomyiidae	01	05	
8.	Coleoptera				2.08
		Braconidae	01	01	
		Staphylinidae	03	05	
		Curculionidae	02	06	
		Coccinelidae	02	11	
		Chrysomelidae	04	13	
		Haliplidae	01	01	
		Erotylidae	04	04	
		Carabidae	01	02	
		Silphidae	01	04	
		Larridae	01	01	
	Unidentified Insects/Insect parts			13	0.56

TABLE 2. Composition of the five clusters of sampling stations in north Arabian sea, which yielded variable number of airborne insects. Cruise III, ORV *Sagar Kanya*, March–April 1996

Sl. No.	Cluster	Sampling Stations	Location
1	I	2, 5, 6, 7, 8	Lat 17 °20′N to 18 °20′N Long 70 °30′E to 71 °30′E
2	II	3, 4, 14, 15	Lat 18 °20′N to 19 °N Long 72 °E to 73 °E
3	III	9, 10, 11, 17, 18	Lat 19 °N to 20 °N Long 70 °E to 71 °30′E
4	IV	19, 22, 29, 30, 32	Lat 20 °10′N to 21 °10′N Long 68 °30′E to 69 °E
5	V	23, 24, 25, 26, 27	Lat 19 °N to 19 °40′N Long 98 °20′E to 69 °E

and Diptera (24%). Coleoptera, Neuroptera, Trichoptera, Lepidoptera and Orthoptera together constituted the remaining 3%. The insects were collected over 24 out of a total of 32 sampling stations, with the remaining stations drawing a blank. While a

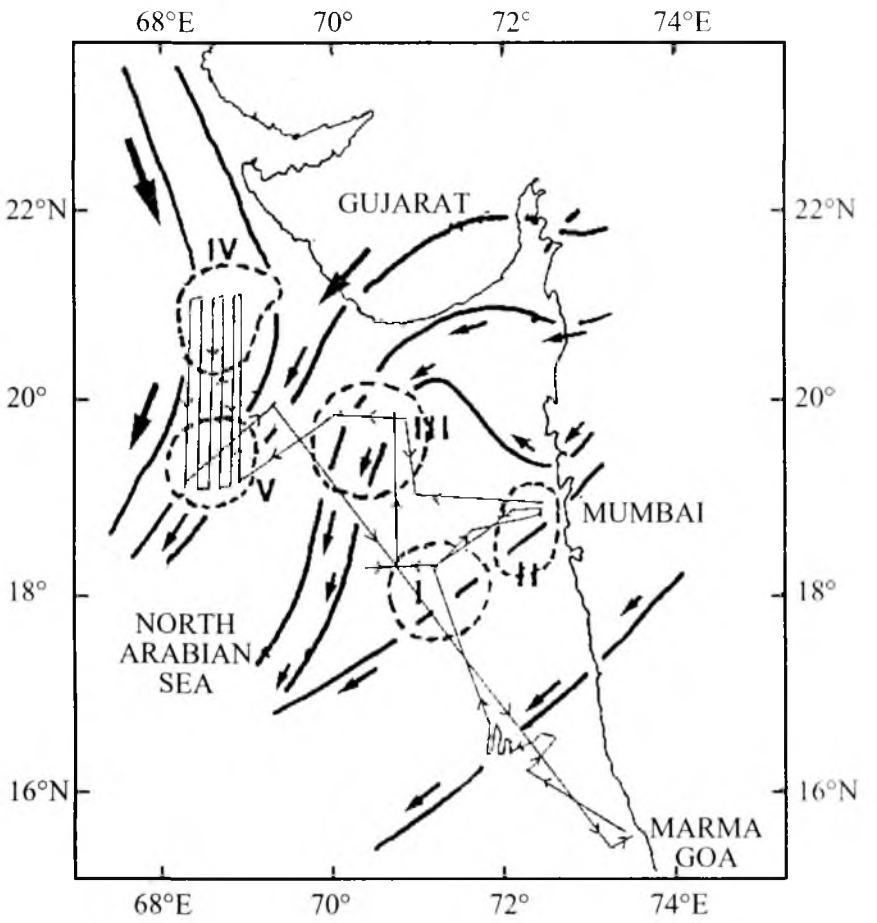

FIGURE 1. Track of cruise #111, ORV *Sagar Kanya* in north Arabian sea during March April 1996. Clusters I to V represent groups of sampling stations with relatively similar aerial density of insects. Superimposed is the generalised wind flow pattern of the region, during the period of study.

large number of insects were recovered at some stations, no insects were recovered at others. The aerial density of insects thus showed variation between groups of sampling stations. The stations were therefore grouped in five clusters, based on contiguity and the differences in the number of insects trapped (Table 2). The location of the clusters with the wind flow pattern in the area (Figure 1) revealed that while the first three clusters received winds from the Indian main land, cluster V received winds both from the Indian main land as well as Persian gulf area. Cluster# IV appeared to receive winds only from the latter source.

Analysis of the data is summarised in Table 3. Four clusters had Agaontidae

TABLE 3. Analysis of the trapping of airborne insects in north Arabian sea. ORV *Sagar Kanya*. March–April 1996

Sl. No.	Clusters	Aerial density in no. $\times 10^4/km^3$	Total number of individuals of all species N(±S.D)	Species			Dominant Family (*pi max*)
				Number detected S	Diversity H	Evenness J	
1.	I (100)*	18.7	399 ± 9.9	49	3.43	0.88	Agaontidae (Hymenoptera)
2.	II (25)	222.5	731 ± 21.5	98	3.93	0.86	-do-
3.	III (225)	77.4	390 ± 13.2	58	3.62	0.89	-do-
4.	IV (600)	60.3	130 ± 8.1	28	3.10	0.93	-do-
5.	V (800)	101.5	189 ± 13.3	44	4.74	0.98	Fulgoridae (Homoptera)

*Figures in parenthesis show the average distance to land *in wind direction* in km.

(Hymenoptera) as the dominant family while Fulgoridae (Homoptera) dominated cluster V. Cluster #II which was closest to the shore had the highest aerial density, number of individuals, number of species and species richness with a slightly lower evenness. Since the winds flew from the north–east (Figure 1), these figures reflected a similar richness on the land from where these winds commenced their south–westward flow. Clusters I and III were similar to each other in as far as the total number of insects, number of species, species diversity and species evenness, with cluster III having an edge. Cluster III however, had aerial density more than four times that of cluster I. Species richness was also distinctly higher in cluster III, as compared to that in cluster I. Cluster #IV which received winds only from the north west (Persian Gulf area) had the lowest number of individuals, number of species, species diversity and species richness, though the figure for species evenness was higher than what had been noted for the other three clusters. There could be many causes for these lower figures. These include richness of species on the land of origin, weather conditions on the land at the time of these insects becoming air borne and of course, the distance over which these insects had travelled with the winds. A good proportion of these insects may even have drowned in the sea waters on their way. We do not have data on these different causes, but we do have data regarding the distance, wind speed and wind direction. The aerial density of insects which reflected this density in the land of origin of these insects *viz.*, the Persian Gulf, was lesser than that seen in cluster III but atleast three times that seen in cluster #1. Cluster V which is located south to cluster IV and is atleast 200 km further away from the nearest land *in wind direction*, had higher number of insects, higher number of species and higher species diversity

besides a distinctly higher aerial density. One possible explanation for this appears to be that stations in cluster V received winds from two sources i.e. the Persian Gulf in the north west as well as the Indian main land in the east. High species diversity and almost total evenness indicate that all species present in trappings in this cluster made similar contribution to the aerial density of insects in the area.

Since this type of work has not been reported so far, it is not possible to compare this data. The only other trapping of airborne insects of terrestrial origin in slightly southern area of Arabian sea was made by Pathak (1998) in which, unlike the present case, Hemiptera was the predominant order (56.3%), followed by Lepidoptera (16.5%), Odonata (10.7%), Diptera (4.9%), Trichoptera (5.8%) and Coleoptera; Neuroptera and Dictyoptera together constituted the remaining 5.8%. But this collection, made in the months of October and November, reflected insects flying towards the Indian shores, unlike the present case when they were flying in the reverse direction. Also this collection did not have specialised insect trapping equipment used in the present investigation. This explains the lower content of smaller dipterans and the complete absence of hymenopterans.

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Effect of Certain Insecticides and Fungicides on the Conidial Germination of *Nomuraea rileyi* (Farlow) Samson

C. Gopalakrishnan* and K. S. Mohan

Division of Entomology and Nematology, Indian Institute of Horticultural Research, Hessaraghatta, Bangalore 560089, India

ABSTRACT: The insecticides, monocrotophos, phosphamidon and dimethoate were highly safe to the entomopathogenic fungus, *Nomuraea rileyi* at all the three concentrations tested. The mean percentage conidial germination recorded in all the three concentrations ranged between 75.91–80.66%. Though, quinalphos, carbaryl, endosulfan and fenvalerate were safe at low concentrations (0.025, 0.050, 0.035 and 0.005% respectively), they were highly detrimental to the fungus at higher concentrations (0.075, 0.150, 0.105 and 0.015% respectively), by way of inhibiting the conidial germination. Similarly, the fungicides, Captofol, Zineb, Chlorothalonil, Fosetyl Al and Ziram, were safe to the fungus at all the three concentrations tested, where the mean percentage conidial germination ranged from 65.1 to 70.3%. Though the fungicides Captan and Sulphur allowed conidial germination at low (0.1%) and normal (0.2%) concentrations (61.0 to 69.75% conidial germination), at high concentration (0.3%) there was total inhibition of conidial germination.

KEYWORDS: *Nomuraea rileyi*, toxicity, insecticides, fungicides, conidial germination.

INTRODUCTION

The entomopathogenic fungus *Nomuraea rileyi* (Farlow) Samson (Deuteromycetes : Moniliales) is an important natural control agent of many lepidopterous insects throughout the world (Fuxa, 1984). The potential of this fungus is being exploited in controlling several noctuid pests (Ignoffo, 1981). Insecticides and fungicides are known to reduce the growth, sporulation and germination of several entomopathogenic fungi (Hall and Dunn, 1959; Yendol, 1968; Cadatal and Gabriel, 1970; Wilding, 1972). As cited by Soper and Ward (1981), agrichemical compatibility is a general concern while entomopathogenic fungi are being developed for crop protection. Here, at Indian Institute of Horticultural research Farm, Bangalore, India, this fungus is being tested for its field efficacy on *Helicoverpa armigera* Hub., *Spodoptera litura* Fab.

*Corresponding author

and *Trichopulusia ni* L. on crops like tomato, cabbage and beet root. The important question often asked is whether *N. rileyi* can be used along with insecticides and fungicides, which are commonly used on tomato and other vegetable crops? Keeping this in view, an experiment was conducted in the laboratory to screen some of the insecticides and fungicides, which are commonly used on tomato to control insect pests and diseases, for their toxicity on the conidial germination of *N. rileyi*.

MATERIALS AND METHODS

The insecticides and fungicides which are commonly being used for the control of insect pests and diseases of tomato were tested for the inhibitory effect on germination of conidia of *N. rileyi*, in terms of colony growth, under *in vitro*. The fungal conidia has been tested for the toxicity of chemicals, as it is the most effective propagule which is being used in the field against susceptible insect pests on several crops.

The insecticides and fungicides used in this experiment are listed in Table 1. The chemicals each at three different concentrations were tested in a simple randomised block design with four replications each. The low concentration was 50% of the normal field application dose, whereas the high concentration was normal field dose plus 50% of the field dose. The normal or medium dose was same as that of field recommended dose (Monocrotophos 0.05%, Phosphamidon 0.5%, Dimethoate 0.04%, Quinalphos 0.05%, Carbaryl 0.1%, Endosulfan 0.07%, Fenvalerate 0.01%, Captofol 0.2%, Zineb 0.2%, Chlorothalonil 0.1%, Fosetyl Al 0.2%, Ziram 0.3%, Captan 0.2% and Sulphur 0.2%).

Preparation of the media and incorporation of the chemicals

33 g of Subouraud maltose agar + yeast (SMAY) (Hi-media, Pvt. Ltd., Bombay, India) was dissolved in 500 ml of distilled water and allowed to boil. Twenty ml of the medium was poured in individual culture tubes of size 15.0×2.3 cm and autoclaved for 30 min at 15 p.s.i. pressure at 121 °C temperature. After cooling but before solidifying, 1.0 ml of the insecticide/fungicide of known concentration was dissolved into the medium. The medium was shaken until an even suspension was obtained and poured into Petri dishes of size 10.0 × 1.3 cm under aseptic condition inside the laminar flow, and the medium was allowed to solidify.

Inoculation of the media with spore suspension

Conidial suspension of known concentration (1.0×10^3 conidia/ml) was prepared through serial dilutions from the stock suspension obtained from the fungal culture maintained on SMAY slants. Counting of spores was done using double ruled improved Neubauer haemocytometer under phase contrast microscope. 1.0 ml of the conidial suspension per plate was poured on to the medium with the help of sterile pipette under aseptic condition. The plates were tilted all around the medium to get uniform seeding of the conidia. The media free from insecticide/fungicide and inoculated with the conidial suspension, as described above, served as control check. The plates were incubated under room temperature of 27 ° ± 2 °C.

TABLE 1. Effect of insecticides on the conidial germination of *Nomuraea rileyi*

Insecticides	% germination of conidia over control*			Mean
	Low concentration	Normal concentration	High concentration	
1. Monocrotophos (Nuvacron 40 SC)	82.75 (65.39)	75.50 (60.27)	69.50 (56.36)	75.91 (60.67)
2. Phosphamidon (Dimecron 100)	85.00 (67.22)	77.25 (63.10)	72.75 (58.42)	78.50 (62.92)
3. Dimethoate (Rogor 30 EC)	89.75 (71.27)	81.50 (64.39)	70.75 (57.12)	80.66 (64.26)
4. Quinalphos (Ekalux 25 EC)	77.75 (61.71)	00.00 (00.00)	00.00 (00.00)	25.92 (26.25)
5. Carbaryl (Sevin 50 WP)	78.75 (61.79)	00.00 (00.00)	00.00 (00.00)	26.25 (20.59)
6. Endosulfan (Thiodon 35 EC)	79.25 (62.86)	00.00 (00.00)	00.00 (00.00)	26.41 (20.95)
7. Fenvalerate (Sumicidin 20 EC)	78.75 (62.70)	00.00 (00.00)	00.00 (00.00)	26.25 (20.59)
Mean	81.71 (64.70)	33.46 (26.82)	30.43 (24.56)	

(Figures in parenthesis are arc sine transformed values)
*Mean of four replicates

	C.D. ($P = 0.05$)	S.Em.
Insecticides	1.96	0.69
Concentrations	1.29	0.45
Interaction	3.40	1.20

Observations recorded

The number of colony growth observed in 3–5 days after inoculation of the medium with the conidial suspension was recorded and the percentage spore germination was calculated.

RESULTS AND DISCUSSION

It has been shown experimentally (Table 1) that the insecticides monocrotophos, phosphamidon and dimethoate were safe to the the fungus as they permitted a mean of 75.91 to 80.66% conidial germination in terms of colony growth at all the three concentrations tested. Urs *et al.* (1967) and Easwaramoorthy and Jayaraj (1977) however, noticed in their toxicity tests against *M. anisopliae, B. bassiana* and *V. lecanii* that phosphamidon showed least inhibition of radial growth. Whereas, neem oil and

neem seed kernel extract proved to be most deleterious to the germination of spores of entomogenous fungus (Devaprasad *et al.*, 1990).

The commonly used insecticides, endosulfan along with quinalphos, carbaryl and fenvalerate were found to be more toxic at higher concentrations. At low concentration the mean percentage of conidial germination ranged between 77.75 and 78.75% (Table 1).

According to Easwaramoorthy and Jayaraj (1977) the insecticides monocrotophos, quinalphos and dimethoate had lesser inhibitory effect on the growth of the fungus *V. lecanii*. In the present study though monocrotophos and dimethoate were safe to *N. rileyi* at all the three concentrations, the insecticide quinalphos was highly inhibitory at normal and high concentrations, whereas at low concentration it was safe.

Thus, it is clear from the above result that the toxicity of insecticides differs for fungal mycelial growth and spore germination. Though most of the work, except a few, was concentrated on toxicity of chemicals towards mycelial growth, it is appropriate of having studied the effect of chemicals on spore germination, because the spores of entomopathogenic fungus are ultimately used in the field to control insect pests.

Results also revealed that the fungal spores of *N. rileyi* can be safely applied in the field along with the insecticides tested when they are used on tomato and other crops to control susceptible insect pests at recommended field dose except the insecticide quinalphos, carbaryl, endosulfan and fenvalerate which can be safely used only at low dose.

Whereas, the fungicides, Captofol, Zineb, Chlorothalonil, Fosetyl Al and Ziram, were safe to the fungus *N. rileyi* at all the three concentrations tested, where the mean percentage conidial germination ranged between 65.1 and 70.3 per cent (Table 2). Though the fungicides Captaf and Sulphur allowed conidial germination at low and normal concentrations (61.0 to 69.75%), at high concentration there was total inhibition of conidial germination (Table 2). Hall and Dunn (1959) studied the effect of five fungicides on the growth of six species of *Entomophthora* and found that Bordeaux mixture was the most harmful while Sulphur was the innocuous. Easwaramoorthy and Jayaraj (1977) in their study on toxicity of fungicides on radial growth of *V. lecanii* showed that the fungicides Bordeaux mixture, Dithane M-45 and Dithane Z-78 were found to be highly harmful recording 67.0–85.1 per cent reduction in growth at all the three concentrations tested. On the other hand Sulphur was found to be less harmful with only 7.6 per cent growth reduction.

Majchrowicz and Poprawski (1993) studied the effect of various fungicides against several entomopathogenic fungi. According to them dithiocarbomate derivatives zineb + copper oxychloride and Mancozeb completely inhibited germination of *Paecilomyces farinosus*. In a few combination, fungus partially overcame or even recovered from the initial inhibition of growth which might have resulted from delayed germination.

Thus, all the fungicides tested can be used safely along with the fungal pathogen at recommended field concentrations with out much affecting the conidial germination of *N. rileyi*. This, however, needed to be confirmed under field situations also. All

TABLE 2. Effect of fungicides on the conidial germination of *Nomuraea rileyi*

Fungicides	% germination of conidia over control*			Mean
	Low concentration	Normal concentration	High concentration	
1. Captofol (Foltaf 80 WP)	75.50 (60.23)	68.75 (55.87)	51.25 (45.60)	65.17 (53.90)
2. Zineb (Dithane z-78)	75.00 (59.97)	68.50 (55.74)	60.25 (50.78)	67.92 (55.49)
3. Chlorothalonil (Kavach 75 WP)	83.50 (65.96)	67.25 (54.95)	58.75 (49.91)	69.83 (56.94)
4. Fosetyl Al (Alliette 80 WP)	77.50 (61.54)	73.25 (58.74)	51.75 (45.88)	69.25 (56.67)
5. Ziram (Cumon L 30 EC)	77.50 (61.54)	69.25 (56.18)	64.25 (53.16)	70.33 (56.96)
6. Captan (Captaf 50 WP)	66.25 (54.38)	61.00 (51.23)	00.00 (00.00)	42.41 (56.96)
8. Sulphur (Sulfex 80 WP)	69.75 (56.53)	65.25 (53.17)	00.00 (00.00)	45.00 (36.57)
Mean	75.75 (60.57)	67.61 (55.13)	40.89 (35.05)	

(Figures in parenthesis are arc sine transformed values)
*Mean of four replicates

	C.D. ($P = 0.05$)	S.Em.
Fungicides	1.81	0.64
Concentrations	1.19	0.42
Interaction	3.14	1.11

though the chemicals mentioned above are safe, it is better if they are applied in a sequence. Timing of the application of chemicals before or after the application of fungal pathogen is very important.

Eventhough, certain insecticides and fungicides were toxic to fungal growth and conidial germination, but as days progresses after contamination of the media (SMAY) the toxicity level goes down. Which necessarily reveals that the chemicals since lose their toxicity as days progresses, the fungus can be applied in the field after a gap of certain period. This needs more study under field conditions.

Studies on the safe interval between the use of pesticides and the entomopathogenic fungi in field is very important because the fungicides used to control phytoparastic fungi might limit the development of fungal mycoses in insect pest population as has been observed by Fisher and Griffiths (1950) in the case of scale insects in citrus. According to Benz (1971) the laboratory tests conducted on artificial media may not give more that tentative answers concerning the influence of insecticides in the living

system. An interval of one weak between the Bordeaux and fungal spray did not impair the pathogenicity of *V. lecanii* against *C. viridis* on citrus has been reported by Easwaramoorthy *et al.* (1977). The inhibitory effect of the toxicants observed on the fungus under controlled conditions was not found under field conditions as observed from the high mortality of the bugs due to fungal infection. Weather elements in the field might act on the toxicants resulting in the metabolic degradation and loss of the toxicants to some extent (Easwaramoorthy *et al.*, 1978).

According to Easwaramoorthy *et al.* (1978) when the toxicants and the fungus applied over larger areas of foliage, the close proximity between the toxicants and spores provided in an artificial medium will not be found in the field. Hence the spores that germinate at a latter time actually come into contact with a reduced dose of the toxicant which might act as a potentiating factor apart from weakening the insect, thereby making the insect most vulnerable to the fungus.

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Drypetes oblongifolia (Bedd.) Airy Shaw: A New Host Record for the Plain puffin, *Appias indra shiva* Swinhoe (Lepidoptera : Pieridae) from Western Ghats, India

C. F. Binoy and George Mathew*

Division of Entomology, Kerala Forest Research Institute, Peechi, Kerala 680653, India
Email: mathew@kfri.org

ABSTRACT: *Drypetes oblongifolia* is reported as a new host plant of the Plain puffin, *Appias indra shiva* Swinhoe at New Amarambalam in the Kerala part of Western Ghats. Adults of *A. indra* were also observed to feed at the flowers of *Syzygium benthamianum*, *S. lanceolata*, *Sapindus trifoliata*, *Leea indica* and *Gordonia obtusa*.

KEYWORDS: Butterfly, Plain puffin, *Appias indra shiva*, *Drypetes oblongifolia*, Western Ghats, Kerala, India.

The Plain puffin, *Appias indra shiva* Swinhoe protected under Schedule II of the Indian Wildlife Act, 1972 (Anonymous, 1990) is a rare butterfly found in Southern India and Sri Lanka (Larsen, 1987). The insect reported to be present in the tropical evergreen zone of the Western Ghats is wide spread in the forests and open areas lying above and below this zone (Wynter-Blyth, 1957).

During a study on insect diversity of New Amarambalam forests of the Nilgiri Biosphere Reserve, a few caterpillars of *A. indra* were collected from *Drypetes oblongifolia*, a widely distributed evergreen tree of Western Ghats. The larvae were found feeding on the upper surface of the newly flushed leaves. The caterpillars of *A. indra* measuring 3.5 cm in length were leafy-green with faint black dots on the dorsal side. The creamy white pupae were laterally flat and had 3 pairs of pointed projections posterior to the head. The hanging pupae were supported by a loose silken body-band attached to the lower surface of the leaf. The adults were noticed to feed on the flowers of *D. oblongifolia*, *Syzygium benthamianum*, *S. lanceolata*, *Sapindus trifoliata*, *Leea indica* and *Gordonia obtusa*. The insect was identified by reference to material available in the KFRI collections and literature (D'Abrera, 1982). This is the first record of *D. oblongifolia* as a host of *A. indra*.

*Corresponding Author

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Susceptibility of *Culex bitaeniorhynchus* Group of Mosquitoes to Two Species of Gregarine Parasites

D. T. Mourya* and R. S. Soman

National Institute of Virology, 20–A, Dr. Ambedkar Road, Pune 411001, India
Email: icmrniv@icmr.niv.ren.nic.in

ABSTRACT: Susceptibility of *Culex bitaniorhynchus* group of mosquitoes to *Ascogregarina culicis* and *A. taiwanensis*, gregarine parasites of *Aedes* mosquitoes was determined. The parasites were found to cause high mortality and did not complete their development in these unnantural hosts. Average survival of infected mosquitoes was also reduced.

Development of resistance to chemical insecticides and their deleterious effects on the ecology has led to a concept of replacing or supplementing them with biological control agents. The present communication reports some of the laboratory observations on the susceptibility of two of the parasites, which naturally infest *Aedes* mosquitoes to the *Culex bitaeniorhynchus* group of mosquitoes.

The parasites *Ascogregarina (=Lankesteria) culicis* and *A taiwanensis* were obtained from infected *Aedes aegypti* and *Ae. albopictus* mosquitoes colonies respectively, maintained at this Institute. *Cx. bitaeniorhynchus*, *Cx. ambiguous* and *Cx. infula* mosquitoes were obtained from separate clean colonies maintained at this institute. First instar mosquitoes larvae were exposed to 10-fold dilutions of oocysts of parasites, which ranged from 10^2 to 10^5 occyst/ml. Infection in the larvae and adult mosquitoes was determined by dissecting out the midguts and malphighian tubes as described by Dhanda and Mourya (1981).

Results showed that all the three species of mosquitoes showed increase in larval mortality with both the parasites in dose dependent manner of oocysts (Table 1). The larvae which survived at a does of 10^4 showed parasite only in about 1–2%. These infected larvae showed occasionally one or two trophozoites of these two parasites. The adults emerging from the surviving larvae were free of *Ascogregarina* infection. In some of the adult mosquitoes dead trophozoites were seen. At a does of 10^5 oocysts/ml there was 100% mortality; no larvae or adults were available to detect parasite development.

Among these two *Ascogregarina* parasites *A. culicis* seems to be more pathogenic that *A. taiwanensis* at a does of 10^3 and 10^4 oocysts/ml. The susceptibility levels of these three species of mosquitoes are *Cx. bitaeniorhynchus* > *Cx. ambiguous* > *Cx.*

*Corresponding author

TABLE 1. Percent larval mortality caused by two parasite species in three species of mosquitoes

Oocysts concentrations	Percent larval mortality		
	C. bitaniorhynchus	*C. ambigous*	*C. infula*
A. culicis			
0/ml	26	26	36
100/ml	53	45	38
1000/ml	90	81	63
10000/ml	87	88	85
100000/ml	100	100	100
A. taiwanensis			
0/ml	24	15	30
100/ml	20	29	31
1000/ml	32	29	44
10000/ml	100	100	47
100000/ml	100	100	100

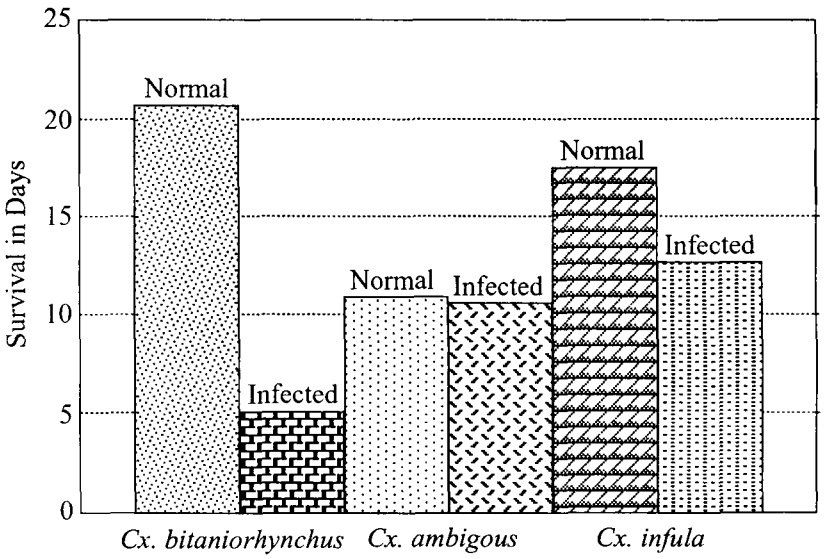

FIGURE 1. Average survival of three *Culex* species of mosquitoes after exposure to parasite *A. culicis*.

infula. Since there was high mortality in these species of mosquitoes at higher dosages, to determine the effect of parasite on the survival of mosquitoes, the mosquitoes were infected with 10^3 oocysts/ml of *A. culicis*. Average survival of the adults that emerged from the larvae which were exposed to oocysts of the parasites was studied. The control batch was treated with triturated suspensions of normal uninfected mosquitoes

of the respective species. Results showed that there was a reduction in the average survival period of *Cx. bitaeniorhynchus*, it was moderate in *Cx. infula* while no effect of parasite was seen on *Cx. ambiguous* mosquitoes (Fig. 1).

The inability of the adults of *Culex* genera to become infected with either of these two parasites supports the (Vavra, 1969) view that different species of *Ascogregarina* have evolved in different hosts of the genus *Aedes*. These parasites have retained some ability to infect other species of mosquitoes of only the same or closely related genera, as observed earlier (Dhanda and Mourya, 1984). Though there was heavy mortality in these *Culex* mosquitoes these parasite species do not complete their development in these hosts hence these parasites have no importance as far as mosquito control is concerned.

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Observations on the Transovarial Transmission of NPV of Silkworm *Bombyx mori* L.

R. Sugun* and P. B. Vijayakumar

Karnataka State Sericulture Research and Development Institute, Bangalore 62, India

Grasserie or jaundice caused by nuclear polyhedrosis virus (Baculoviridae) is a serious disease inflicting considerable cocoon loss to the sericulturists (Sidhu and Singh, 1968). Vaidhya (1960) reported that the cocoon yield loss due to grasserie disease of silkworm accounts to 15 percent. The sericulturists were of the opinion that like the pebrine disease of silkworm, the grasserie was also transmitted transovarially and caused heavy damage to the crops in summer and rainy seasons. As there is no such information available, laboratory experiments were conducted using freshly moulted fourth instar larvae and fed them with 2.5×10^6, 5×10^6 and 10×10^6 polyhedral Inclusion Bodies/ml. The pathogen load was smeared on mulberry leaves and the silkworms were allowed to feed on such treated leaves for 24 h. In control, sterile distilled water alone was used instead of virus inoculum. Observations were made daily on the larval mortality due to NPV infection and other causes, larval and pupal duration.

After the cocoon formation the male and female pupae were collected, moths allowed to mate for 24 h. Layings were prepared for immediate rearing activities. Results form three experiments showed that the hatching varied form 90–95 percent. The chawki silkworms were examined daily for the presence of polyhedra through the microscope, which were not seen throughout the development of larval stages. Further, the feeding rate, moulting behaviour, weight of the larvae, spinning process, pupal duration and cocoon formation were very normal when compared to the control. The treated larvae showed neither symptom(s) nor mortality due to polyhedrosis.

This indicated that the NPV of silkworm cannot be transmitted transovarially as in case of the pebrine disease of silkworm.

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*Corresponding author

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Impact of Pesticides on Carabids of Rotational Intensive Cropping Systems

R. Swaminathan* and V. V. Isaichev

Department of Agricultural Zoology & Entomology, Rajasthan College of Agriculture, Udaipur 313001, India

ABSTRACT: Few commonly used pesticides against weeds and insect pests of intensive rotational cropping systems of the central non-chernozomes of Russia were tested for their bio-efficacy against the dominant species of ground beetles in laboratory and mini-field experiments. The herbicides, Dialen and Lontrel were safe to the beetles as no mortality was recorded. Among insecticides, Basudin was most toxic to all the four dominant species (*Poecilus cupreus* L., *Pterostichus melanarius* (Ill.), *Pseudoophonus rufipes* DeG., and *Harpalus affinis* (Sch.) in the laboratory (LC_{50}: ranging from 0.000226 to 000744%), and in the field trials (mortality after 7 days ranging from 80 to 100%). Fenvalerate was least toxic to all the species in the laboratory (LC_{50}: ranging from 0.00729 to 0.01%). In the mini-field trials, the synthetic pyrethroids responded according to the species. Cypermethrin was more toxic to the carabids; Decamethrin was relatively safe to *P. melanarius* and *P rufipes* while less toxic to *H. affinis* and *P. cupreus*.

KEYWORDS: Pesticide, carabids, rotational cropping system.

Carabids are important components of arable lands because many species are predators of phytophagous insects (Kirk, 1975; Best and Beegle, 1977a,b; Lund and Turpin, 1977; Los and Allen, 1983; Isaichev and Swaminathan, 1993), and they have a positive impact in reducing pest abundance (Hance, 1987). Ground beetles are very susceptible to insecticides and other environmental pollutants (Kulman, 1974). Reductions in their abundance and diversity have been shown after use of insecticides in various cultivated crops (Herne, 1963; Dunning *et al.*, 1975; Hsin *et al.*, 1979; Dritschillo and Wanner, 1980; Los and Allen, 1983; Basedow *et al.*, 1985).

The influence of six commonly used pesticides, Basudin 60 EC, Decamethrin 2.5 EC, Cypermethrin 25 EC, Fenvalerate 20 EC, Dialen 40 EC and Lontrel 52.5 EC, was studied on the ground beetles. Adult carabids were collected from biologically managed intensive rotational cropping systems of the central non-chernozome Podolsk region, located on the outskirts of Moscow, Russia, during summer 1991. Glass containers (500 ml.) with a mouth diameter of 8 cm. were placed in pits 15 metres apart and leveled to ground surface. The pitfall traps were covered with a plastic cone

*Corresponding author

affixed to stands of wire. This was done to avoid rainwater from entering into the traps and to safeguard the collected carabids from predators.

Adult beetles of the four dominant species [*Poecilus cupreus* L.; *Pterostichus melanarius* (III.); *Pseudophonus rufipes* (DeG.); and *Harpalus affinis* (Sch.)] were maintained in the laboratory for one week before the bioassay. The beetles were kept separately in glass jars (30 cm. diameter) with a layer of moist soil and were provided food (pieces of soft beef and bread crumbs) and a dilute solution of sucrose contained in a cotton swab placed in a petridish. No cannibalism was observed.

From a stock solution of 1 percent, the pesticide dilutions made were as: Basudin: 0.0001, 0.0003, 0.0006, 0.0009 and 0.0012; Decamethrin, Cypermethrin and Fenvalerate: 0 001, 0.003, 0.006, 0.009 and 0.012; Lontrel and Dialen: 0.05, 0.1, 0.15, 0.2 and 0.25 percent in acetone. The dry-film residue technique was used for the bioassay pouring 1 ml from each aliquot into petridishes of 10 cm diameter. Each concentration was replicated five times and a control with pure acetone was run side by side. Ten healthy adult beetles were released in each replicate, exposed for 30 minutes and thereafter transferred to clean jars containing moist soil with grass and food. Observations on mortality were taken 24 hours after the treatments. The data thus obtained were subjected to Probit analysis.

To ascertain the effect of these commonly used pesticides on the dominant carabid fauna under field conditions, a mini-field experiment was conducted in 1991 at the Departmental Research Station of Moscow Agricultural Academy, Moscow. Plots of 1 square metre were prepared. Recommended doses (in ml./sq.m.) of the six aforesaid pesticides were used: Basudin (0.09 and 0.18); Decamethrin (0.025 and 0.05); Cypermethrin (0.016 and 0.032); Dialen (0.2) and Lontrel (0.3). After treatment, four metallic cylinders (mouth diameter 15.5 cm and height 25 cm) with open bottoms were inserted into the moist soil of the treated plots. A parallel control plot was also maintained with water spray. The soil enclosed in each cylinder had a volume of 2926 cub cm. To each of these metallic cylinders, another cylinder made of thick plastic sheet was attached to raise the height 15 cm more so that the beetles did not climb out. Four such cylinders were placed in each plot of 1 sq m to which 10 healthy adults were released. Food was provided soon after release. Observation on mortality was recorded 1, 3 and 7 days after treatment and the data obtained were subjected to ANOVA.

The herbicides, Dialen and Lontrel, had no toxic action on the carabids therefore, have been excluded from the tables. Fenvalerate was least toxic, while Basudin was most toxic to all the four dominant species (based on LC_{50} values). However, each of the four species responded differently to the insecticides when compared with Fenvalerate (Table 1). In the mini-field experiment also the herbicides were non-toxic to the carabids. Among the insecticides, Basudin was most toxic to all the four species at the recommended dose (0.18 ml/m^2) and even half the dose (0.09 ml/m^2) after 24 hours. The mortality recorded after 7 days ranged from 80 to 100 percent depending upon the species [Tables 2(A)–(D)]. Basedow *et al.* (1985) observed that Deltamethrin sprays (7.5 g. a.i./ha in 300 litres of water) on winter rape and wheat did not affect the

TABLE 1. Comparative toxicity of insecticides to dominant carabids

Insecticides (EC)	LC50	Fiducial Limits	Regression equation	Chi-square	Relative toxicity
(A): *Poecilus cupreus*					
Basudin 60	0.000744	0.000887 0.000825	$Y = 2.73 + 2.6x$	30.504	10
Decamethrin 2.5	0.00547	0.00624 0.00479	$Y = 2.44 + 3.46x$	6.562	1.3
Cypermethrin 25	0.00625	0.00905 0.00431	$Y = 1.93 + 3.85x$	5.62	1.2
Fenvalerate 20	0.00729	0.0088 0.00604	$Y = 1.82 + 3.69x$	2.245	1
(B): *Pterostichus melanarius*					
Basudin 60	0.000226	0.000246 0.000208	$Y = 2.35 + 7.46x$	4.201	79
Decamethrin 2.5	0.00372	0.00483 0.00287	$Y = 1.98 + 1.92x$	9.453	5
Cypermethrin 25	0.00474	0.00583 0.00384	$Y = 0.76 + 2.53x$	3.214	4
Fenvalerate 20	0.0178	0.0213 0.0149	$Y = 1.55 + 2.75x$	5.072	1
(C) *Pseudophonus rufipes*					
Basudin 60	0.000394	0.000462 0.000335	$Y = 3.15 + 3.10x$	2.263	28
Decamethrin 2.5	0.00432	0.00527 0.00354	$Y = 0.5 + 2.75x$	7.273	2.5
Cypermethrin 25	0.00763	0.00863 0.00674	$Y = 0.96 + 4.57x$	4.107	1.5
Fenvalerate 20	0.011	0.0135 0.0091	$Y = 1.49 + 3.36x$	1.378	1
(D) *Harpalus affinis*					
Basudin 60	0.000366	0.000389 0.000343	$Y = 2.45 + 4.52x$	4.233	22
Decamethrin 2.5	0.00474	0.00586 0.00382	$Y = 0.95 + 2.41x$	4.184	1.7
Cypermethrin 25	0.00416	0.00525 0.00629	$Y = 1.41 + 2.22x$	6.574	2
Fenvalerate 20	0.00801	0.00874 0.00734	$Y = 2.15 + 3.16x$	5.594	1

TABLE 2. (A) Insecticidal efficacy on *Poecilus cupreus* (Mini-field experiments)

Treatments	Dose ml per sq m	Percent mortality after 1 day	3 days	7 days
Basudin	0.09	60.09 (50.83)	70.23 (56.94)	80.50 (63.90)
	0.18	96.19 (78.75)	99.35 (85.39)	100.00 (90.00)
Decamethrin	0.025	02.56 (09.21)	02.56 (09.21)	26.21 (30.80)
	0.05	68.33 (55.75)	70.50 (57.10)	88.32 (70.02)
Cypermethrin	0.016	13.92 (21.91)	34.44 (35.94)	55.39 (48.10)
	0.032	70.15 (56.88)	88.65 (70.31)	100.00 (90.00)
C.D. (5%)		18.69	11.87	11.53

TABLE 2. (B) Insecticidal efficacy on *Pterostichus melanarius* (Mini-field experiments)

Treatments	Dose ml per sq m	Percent mortality after 1 day	3 days	7 days
Basudin	0.09	98.66 (83.36)	100.00 (90.00)	100.00 (90.00)
Decamethrin	0.025	0	0	8.01 (16.44)
	0.05	44.86 (52.05)	92.53 (74.14)	97.43 (80.78)
Cypermethrin	0.016	10.85 (19.33)	34.16 (35.77)	39.51 (38.95)
	0.032	65.44 (53.99)	99.35 (85.39)	99.35 (85.39)
C.D. (5%)		17.89	15.42	17.75

four carabid species adversely, but the numbers of three staphylinid species and the liniphyiid spiders were greatly reduced (62.2 and 92 percent mortality, respectively). Cabofuran and Terbufos, commonly used soil insecticides, were reported to have no significant detrimental effect on any pest, parasite or predator population when applied as foliar spray on alfalfa @ 0.14 and 0.56 kg/ha (Surgeoner and Ellis, 1975). On the

TABLE 2. (C) Insecticidal efficacy on *Pseudophonus rufipes* (Mini-field experiments)

Treatments	Dose ml per sq m	Percent mortality after 1 day	3 days	7 days
Basudin	0.09	94.72 (76.11)	100.00 (90.00)	100.00 (90.00)
Decamethrin	0.025	0	0	0
	0.05	24.58 (29.73)	49.99 (44.99)	55.01 (47.87)
Cypermethrin	0.016	0	01.34 (06.64)	05.27 (13.28)
	0.032	60.47 (51.05)	98.66 (83.35)	100.00 (90.00)
C.D. (5%)		12.64	14.25	13.10

TABLE 2. (D) Insecticidal efficacy on *Harpalus affinis* (Mini-field experiments)

Treatments	Dose ml per sq m	Percent mortality after 1 day	3 days	7 days
Basudin	0.09	78.15 (62.14)	85.31 (67.47)	99.35 (85.39)
Decamethrin	0.025	07.46 (15.85)	14.67 (22.52)	29.75 (33.05)
	0.05	45.65 (42.50)	50.15 (45.08)	87.57 (69.35)
Cypermethrin	0.016	24.03 (29.35)	37.22 (37.59)	60.47 (51.05)
	0.032	70.23 (56.93)	85.15 (67.33)	99.35 (85.37)
C.D. (5%)		09.90	08.27	09.29

contrary, Tomlin (1975) found Terbufos and Carbofuran to be highly toxic to the adult carabid, *Agonoderus comma* (F) but less toxic to larval *Pterostichus melanarius* (III.), when tested by contact method. Hsin *et al.* (1979) reported *Pterostichus chalcites* to be more susceptible to Terbufos and Carbofuran than to Dieldrin, possibly due to resistance to Dieldrin. The dominant carabid, *Harpalus pensylvanicus* DeG., was more abundant in untreated alfalfa fields, having a total of 43 spices, than those treated with insecticides, which had 30 species (Los and Allen, 1983). Aldicarb with organic manure did not affect the number of individuals but slightly diminished the number

of species. In contrast, Aldicarb without organic manure did not affect the number of individuals but slightly diminished the number of species. In contrast, Aldicarb without organic manure reduced the number of carabids three-fold. Similarly, low organic manure associated with Parathion and Metasystox reduced the number of carabid species and their abundance (Hance and Gregorie-Wibo, 1987). It is evident that pesticide use in arable lands must be restricted/selective to conserve and augment these non-specific epigeal predators, which are capable of preventing insect pest outbreaks.

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Natural Parasitism on the Pomegranate Hairy Caterpillar *Trabala vishnou* Lefevere (Lepidoptera : Lasiocampidae) in Karnataka

M. Mani*, C. Gopalakrishnan and A. Krishnamoorthy

Division of Entomology and Nematology Indian Institute of Horticultural Research, Hessaraghatta Lake P.O., Bangalore 560089, India

ABSTRACT: The hairy caterpillar *Trabala vishnou* Lefevere appeared in considerable numbers on pomegranate in and around Bangalore in 1998. It was found parasitised by the tachinid *Blepheripa zebina* Walker and also infected with nuclear polyhedrosis virus (NPV) in the field. The per cent larval parasitism went up to 100% but the extent of viral infection did not exceed more than 5% during October–November. Both *B. zebina* and NPV were reported on *T. vishnou* attacking pomegranate for the first time in Karnataka.

KEYWORDS: Pomegranate, *Trabala vishnou*, *Blepheripa zebina*, tachinid parasitoid, Nuclear polyhedrosis virus.

The hairy caterpillar *Trabala vishnou* Lefevere is a sporadic polyphagous pest that occurs throughout India (Rathore and Verma, 1977; Rao and Goel, 1986; Joshi *et al.*, 1988; Gurdip Singh *et al.*, 1990; Khatua, 1997). *T. vishnou* has been reported feeding on the leaves of castor, tamarind, banana, pomegranate, *Rosa* sp. *Syzygium cumini* L., *Eucalyptus botryoides* S., *Shorea robusta* Gaertn. Etc. leaving the veins giving skeletonised appearance (Vishwanath and Gowda, 1974; Rathore and Verma, 1977). In October 1998, *T. vishnou* was first observed on pomegranate at IIHR Farm, Hessaraghatta and then two other locations *viz.*, Yelahanka and M.S. Palya. From all the three localities, the larvae of *T. vishnou* were collected regularly from 10plants and kept them feeding with fresh pomegranate leaves daily until pupation in the cloth walled wooden cages (30 × 30 × 30 cm). The number of adult moths and parasitoids emerged was recorded to work out the per cent parasitism. The identity of the moth was determined at University of Agricultural Sciences, Bangalore and that of the tachinid at Project Directorate of Biological Control, Bangalore.

Field collections of *T. vishnou* had yielded the tachinids, which were identified as *Blepheripa zebina* Walker. The larvae of parasitoids (1–3) had emerged from the pupae of *T vishnou*. The data on per cent parasitism from three locations is given in Table 1. Most of the pupae had yielded the parasitoids and the per cent parasitism ranged from

*Corresponding author

TABLE 1. Natural parasitism by *Blepheripa zebina* on *Trabala vishnou* in pomegranate orchards around Bangalore

Hessaraghatta		Yelahanka		M.S. Palya	
Date	Parasitism (%) (Mean ± SD)	Date	Parasitism (%) (Mean ± SD)	Date	Parasitism (%) (Mean ± SD)
10/10/98	90.91 ± 4.85	5/10/98	80.00 ± 8.15	9/9/98	75.00 ± 4.80
21/10/98	85.71 ± 3.60	16/10/98	100.00	25/9/98	100.00
4/11/98	100.00	5/11/98	88.89 ± 4.20	15/10/98	77.78 ± 3.18
12/11/98	80.00 ± 6.10	17/11/98	66.67 ± 7.12	2/11/98	75.00 ± 5.45
Mean	89.74 ± 3.92	—	85.29 ± 2.92	—	81.82 ± 2.92

SD = Standard Deviation.

85.29% to 100% during October–November. Since the parasitism was high, the pest was not observed from December'98 in these locations.

In the present investigation, only *B. zebina* was observed on *T. vishnou*. It was known to be attacked earlier by dipteran parasitoids *B. zebina* (Crosskey, 1976), *Ugimyia sericariae* (Joshi *et al.*, 1988) and hymenopteran parasitoid, *Mesocomys orientalls* Cam. (Khan, 1983). The leaf damage by caterpillars was found to be negligible in the field in the present study. The less leaf damage might be due to less feeding by the caterpillars parasitised by *B. zebina*. The disappearance of the pest from December might be due to heavy parasitism in the field. Rao and Goel (1986) also reported that dipteran parasitoids constituted the major mortality factors in the third generation of *T. vishnou*.

During study period, some dead caterpillars of *T. vishnou* showing typical 'tree top disease' symptoms, i.e. diseased caterpillars hanging head downwards with prolegs affixed to the twigs, were collected and brought to the laboratory. The body of the diseased caterpillars were soft but unlike the other nuclear polyhedrosis virus (NPV) affected lepidopterous larvae, where the body becomes fragile and breaks easily, the integument was tough. Examination under phase contrast microscope showed large number of poly occlusion bodies. the virus was purified under differential centrifugation and the pathogenicity proved positive to the young caterpillars of *T. vishnou*. There was also report of NPV infection on *T. vishnou* infesting castor in punjab (Battu, 1986) and *Aspergillus flavus* on *T. vishnou* attacking *Eucalyptus* spp. in Assam (Joshi *et al.*, 1988). Vishwanath and Gowda (1974) also reported heavy mortality of *T. vishnou* due to some bacterial disease in Karnataka.

Both *B. zebina* and NPV are reported on *T. vishnou* in pomegranate orchards for the first time in Karnataka. Application of NPV could supplement the heavy parasitism by *B. zebina* in bringing complete suppression of *T. vishnou* on pomegranate.

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